Cigarette smoking and rheumatoid arthritis

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By

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ABSTRACT

Rheumatoid arthritis (RA) is an umbrella term used to describe the condition of individuals who have a chronic (3 months or more) symmetrical polyarthritis (more than 5 joints) usually involving the small joints of the hands and feet. At one end of this clinical spectrum are individuals who are rheumatoid factor (RF) positive and who have joint erosions and at the other end are individuals who never demonstrate these features. Recent studies indicate that RF positivity and cigarette smoking are closely linked in both healthy individuals, and RA patients. Further, extensive epidemiological studies have suggested a link between cigarette smoking and the development of seropositive RA. Clinical studies have also demonstrated a link between prolonged heavy cigarette smoking and the development of high titre seropositivity, the development of rheumatoid nodules and joint damage in RA patients.

To date, research into the link between RA and cigarette smoking has only concentrated on RF production, the presence of rheumatoid nodules, functional disability and joint damage in relatively mild cases found in the community. The conclusions are that smoking has very little impact on the occurrence of RA but is related to severity. However there has been no accurate determination of the amount of cigarettes smoked and no studies have specifically addressed the more severe end of the spectrum as found in patients attending hospital for their rheumatoid disease.

Clinically, it is apparent that heavy cigarette smoking is a risk factor for hospital treated RA and that heavy smoking and non-smoking RA patients differ in a number of ways. Heavy smoking RA patients present in late middle age and are less likely to have a family history of RA than life long non-smoking RA cases that present earlier and have a striking family history of RA.

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The principle aim of this study was to test the hypothesis that heavy smoking is an aetiological factor in RA and generates a distinct subgroup of the disease definable in terms of clinical phenotype, particularly severity. A second aim was to investigate possible molecular mechanisms linking smoking with RA and what I believe to be candidate mechanisms involving the detoxifying glutathione S transferase Mu 1 (GST M1) gene and oxidative damage to alpha 1 proteinase (α1 PI). These studies involved a review of the literature regarding the link between RA and both smoking and α1 PI deficiency. I investigated the relationship between heavy cigarette smoking and hospital based, more severely affected RA patients. Additionally the age of onset and smoking history was compared in familial and sporadic RA cases. Regarding smoking and severity of RA, a cohort of RA patients were studied to determine if smoking was an independent risk factor for severe RA and whether this effect was influenced by the presence (GSTM1-1) or absence (GSTM1-0) of the GST M1 gene. Oxidative damage in RA to the α1 PI protein was studied in relation to rheumatoid disease activity, GST M1 and cigarette smoking. The oxidative damage to α1 PI was measured in terms of serum levels of Immunoglobulin A- α1 PI (IgA-α1 PI).

In summary, I have shown that heavy cigarette smoking is strongly associated with hospital based RA. Secondly that familial RA presents at an earlier age than sporadic RA in individuals smoking at disease onset only and that sporadic RA patients are significantly more likely to smoke at disease onset than familial RA patients.

I have confirmed previous findings that raised serum IgA-α1 PI levels are associated with erosive as opposed to non-erosive RA cases and demonstrated that principally these raised serum complexes occur as a result of cigarette smoking and increased disease activity.

It has also been demonstrated that in RA patients attending hospital outpatients with longstanding disease, cigarette smoking is associated with a worse prognosis in terms of joint

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damage (Larsen score) and disability (HAQ score). Potentially underlying this association was the finding of a greatly increased prevalence and concentration of RF in smokers. A correlation between the number of years smoked and pack years smoked by the patient and RF levels was observed.

When considering the GST M1 gene GSTM1-0 was only clinically relevant in smokers in terms of rheumatoid joint damage. Underlying this was a highly significant association between GST M1-0 smokers and RF production. This is likely to represent an important genetic-environmental interaction in RA.

Finally no association between GST M1-0 smokers and raised serum levels of IgA-α1 PI levels was observed.

Acknowledgements

Ethical Approval

Ethical approval was obtained for all the studies reported herein from the Whiston and St.Helen's and South Sefton Ethics Committees, Merseyside.

Abbreviations

RA	Rheumatoid arthritis
RF	Rheumatoid Factor
ESR	Erythrocyte sedimentation rate
CRP	C reactive protein
GST	Glutathione s Transferase
GST M1	Glutathione s Transferase Mu 1
α1 PI	Alpha 1-Proteinase inhibitor
IgA-α1 PI	Immunoglobulin A- Alpha 1-Proteinase inhibitor
NE	Neutrophil Elastase
MMP	Matrix metalloproteinase
TIMP	Tissue inhibitor of Matrixmetalloproteinase
FH	Family history
COPD	Chronic obstructive pulmonary disease
IHD	Ischaemic heart disease
EIC	Elastase inhibitory capacity.
TNF α	Tumour necrosis factor alpha

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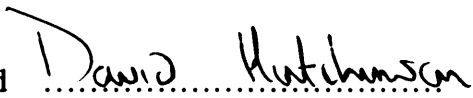
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ABSTRACTS

1. Hutchinson D, R Moots, M. Lynch. **Cigarette smoking is positively associated with development of rheumatoid arthritis (RA) in patients without a family history of RA.** Arthritis Rheum 1999;49(suppl).1054.

2. Hutchinson D, L. Shepstone. R Moots, M. Lynch. **Rheumatoid arthritis (RA) is strongly associated with increased cumulative exposure to cigarette smoke.** Arthritis Rheum 1999;49(suppl).1055.

Disclaimer

All the ideas for the research undertaken in this thesis were my own. All the data and samples for all the patients studied in Liverpool were collected by myself. Dr. J Lear collected the dermatology control case data. The assays for the IgA-α1 PI complex were undertaken by myself and Nicola Nixon at the Haywood hospital Stoke on Trent The statistical work for chapter 2 was undertaken by myself, Dr.Lee Shepstone and Dr. Derek Mattey. Nicola Nixon and Dr. Anthony Fryer undertook the detoxifying genotype work. Dr. Derek Mattey exclusively undertook the collection of data, statistical analysis regarding the patients studied in Stoke-on-Trent.

Signed ....

Date 28th November 2003

Name   David Hutchinson

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Chapter 2

Methods

Chapter 3

**Heavy cigarette smoking is strongly associated with RA
particularly in those without a family history of RA**

Chapter 1

Introduction and background

1.1 The natural history of RA

The first clear description of RA was probably that published by Landre-Beauvais in 1800, who called it "la goutte asthenique primitive." This work was submitted in his MD thesis to the University of Paris (Landre-Beauvais AJ, 1800). He described a new rheumatic disease in nine patients who were long–term residents of Salpetriere hospice in Paris. This disease he termed "asthenic gout" and described several distinctive features, including a predominance in women, a chronic course, involvement of many joints from disease onset and a decline in general health.

In the 19th century a number of English and French physicians described and illustrated the classical features of the disease (Fraser KJ, 1982; Adams R, 1857).

Sir Alfred Baring Garrod (1819-1907) only named the disease in 1859 (Garrod AB, 1859). This work was followed by others and included the famous French neurologist Jean-Martin Charcot (1825-1893). In 1890, he presented a detailed account of the disease, describing characteristic physical findings of the hands and the fingers, including those that were determined "swan neck" (deformations articulaires du premier type), and "Boutonniere" du deuxieme type). These depictions of arthritis are clearly recognisable as RA.

It has been recently suggested that the relatively late description of true RA may reflect the emergence of a contemporary disease (Buchanan WW, 1998). This hypothesis is supported by archaeological investigations that have failed to discover skeletal remains in the Western world before 1800 with convincing changes of RA. There has been no documented evidence of RA in Egyptian mummies in contrast to other common rheumatic diseases, including osteoarthritis, ankylosing spondylitis, gout, chondocalcinosis and the very rare ochronosis (Buchanan WW, 1998).

Furthermore a review of the 18th century bone and joint collections of the two Hunter brothers, William (1718-1783) in Glasgow University and John (1728-1793) in the Royal

College of Surgeons of England in London. Despite a huge collection of bone and joint pathology, including the first case of the rare osteitis fibrosa cystica Buchanan et al, 1981 were unable to describe a single case of RA.

A recent review of the medical literature found no description of classical RA prior to the 19th century (Buchanan WW, 2001). Concerning the fine arts, Talbott in 1981 declared that he was unaware of RA in any painting depicting classical RA. However, Aceves-Avila et al, 2001 give at least four examples of paintings depicting classical RA prior to the 1800s.

However studies of skeletal remains of Native North American Indians suggests a high prevalence of convincing RA dating back thousands of years (Rothschild, BM, 1988; Woods RJ, 1988; Rothschild BM, 1990). Cigarette smoking was embraced by the North American Indian culture approximately 2000 years ago. It has been hypothesised that the late introduction of tobacco to the Western World is a possible explanation for the striking difference in the time of emergence between the New and the Old World (Fischer KM, 1991).

Clearly this evidence suggests that cigarette smoking may be an important trigger for the development of RA. This is certainly the case with other contemporary diseases such as lung cancer and pulmonary emphysema.

Additionally the type of RA treated by rheumatologists in the U.K. has changed over the last four or five decades. Seropositive RA, nodular RA and rheumatoid vasculitis are far less prevalent than forty years ago. This may of course simply reflect a far more successful treatment regime preventing the development of seropositive, nodular and vasculitic RA. However, at presentation the prevalence of seropositive RA has decreased markedly over the last 4 decades (Silman AJ, 1986).

1.1.a The natural history of cigarette smoking in Britain.

An epidemic of cigarette smoking occurred in the UK in the last century. Tobacco use by men was well established by 1841. It increased up to the end of the Second World War, when it fell slightly before stabilising. After the 1960s it fell substantially. Between 1890 and 1950 cigarettes largely replaced other tobacco products. Use by women commenced in the mid-1920s and, with few exceptions, was limited to cigarettes. It increased rapidly in the Second World War and continued to increase until the 1970s when it began to fall.

Smoking by men

It is estimated that in 1871 average annual consumption of all tobacco products per person (aged 15 years or more) was 2.6 Kg. Consumption rose continually until the early 1940s, when it stood at 5.3 Kg per annum. Since the Second World War consumption of tobacco has fallen particularly since the late 1960s, so that by 1987 it was estimated to be 2.4 Kg per annum, slightly lower than in 1871.

The overall amount of tobacco consumed is not a good indicator of disease risk, because different tobacco products are associated with different disease risks. During the nineteenth century most of the tobacco sold was smoked in pipes and to a far lesser extent used for cigars, snuff and chewing. Cigarettes were a rarity, and at the beginning of the twentieth century the average per head consumption of tobacco in the form of cigarettes by men aged 15 years or over is estimated to have been 0.5 kg per annum, accounting for only 16% of total tobacco consumption. During the first part of the twentieth century, however, this increased rapidly so that in the early 1940s it had risen to 4.1Kg, accounting for nearly 80% of all the tobacco that men consumed. Since this time cigarettes have accounted for up to 90% of men's tobacco consumption.

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Until the early 1960s the number of cigarettes smoked per day was a good reflection of the weight of cigarette tobacco smoked per person. The introduction in the 1960s of filter cigarettes meant that there was an actual significant reduction in tobacco consumption by weight, despite the fact that the number of cigarettes smoked remained static. In the late 1970s men were smoking an average of 10.5 cigarettes per day, similar to the value of 10.9 at the end of the 1940s, but consumption in terms of tobacco was almost 30% lower. During the 1980s the number of cigarettes smoked per day by men fell progressively, to an average of 7.3 in 1987. This equates to a fall of over a half in consumption of tobacco in term of weight in the last forty years.

However, cigarette consumption is not the only factor to be considered when investigating cigarette- related disease. Mortality from smoking related diseases was reduced by about 23% for a reduction in tar yield of 15 mg per cigarette. Additionally mortality from ischaemic heart disease was also reduced by 23%, and mortality from lung cancer was reduced by 25% (Tang, 1995). This is not altogether surprising given the strong evidence existing linking increased tar consumption with lung cancer (Hammond, 1976:Wynder, 1979: Hawthorne, 1978: Higenbottam, 1982: Vutuc, 1983: Lupin, 1984: Kaufman, 1989.)

Therefore methods of cigarette manufacture are of importance. Since the 1960s, the tobacco industry has had a policy of reducing the toxicity of cigarettes by reducing the amount of tar delivered when a cigarette is smoked (Waller et al, 1996). This policy was pursued initially through a series of voluntary agreements between government and the tobacco industry, and more recently through European Union directives (Bates et al, 1999). The new directive that has been recently agreed imposes a tar limit of 10 mg per cigarette, nicotine to 1mg per cigarette and carbon monoxide to 10 mg per cigarette.

The earliest figures for the yield of tar per cigarette relate to the late 1930s, and indicate an average value of 33 mg per cigarette. This remained constant until the early

1960s as a result of a switch from plain to filter cigarettes. The policy of reducing tar yields has been successful over the last thirty- five years and in 1999 the tar yield per cigarette was 9.5 mg per cigarette (Jarvis, 2000). This is below the limit of 10 mg that is to be introduced throughout the EU by 2002.

Overall there has been a decline in smoking prevalence amongst men in the UK. Social class is now a very important determinant of smoking prevalence in men. In 1961 before the health implications of smoking were widely known there was very little difference in smoking prevalence between the social classes. Fifty three per cent of men of socio-economic group I (professional class) smoked as opposed to 60% of men of social class V (manual workers). However, there has been a dramatic divergence in the smoking history of these two groups such that in 1991 17 per cent of men of socio-economic group I smoked as opposed to 52% of men of social class V.

Smoking by women

Consumption of tobacco by women has been almost exclusively in the form of manufactured cigarettes. Women did not start smoking on a large scale until the mid-1920s. Thereafter, consumption rose steadily, both in terms of cigarettes per day, until the late 1970s, when women were consuming on average 1.9Kg of tobacco annually and smoking 6.8 cigarettes per day. Since then tobacco consumption has fallen to 1.6Kg per annum or 5.1cigarettes per day in 1987.

When tar weighting is taken into account, consumption by women started to fall about 5 years earlier. The fall has been more substantial than in men, so that in 1987 it averaged 2.2 tar-weighted cigarettes per women per day, about half its peak of 4.5 tar weighted cigarettes per day in the late 1960s.

Although cigarette consumption in women has always been lower than in men, their consumption as a proportion of that in men has been steadily increasing ever since they

started to smoke. In the early 1930s consumption by women in terms of tar –weighted cigarettes per person per day was only 9% of that in men, whereas in the early 1950s it was 30 per cent. In the early 1970s it had risen to 56 per cent and in 1987 stood at around 70 per cent.

1.1.b The role of a family history in RA

Rheumatoid arthritis is a complex multifactorial disease. In such a disease both environmental and genetic factors are likely to play a role in susceptibility to disease. In most RA individuals there will be a complex interaction between environmental factors and genetic factors. The longstanding question of nature or nurture regarding RA susceptibility remains relevant for some individuals with RA as these individuals may have a predominance of either a genetic/familial factor or a strong environmental factor triggering their disease. This thesis is almost entirely devoted to the role of cigarette smoking in RA. However to ignore other fundamental risk factors for disease susceptibility would be a mistake as there is likely to be an interaction or possibly a disassociation between these risk factors and cigarette smoking.

Genetic factors have long been thought to be important in the development of RA. Rheumatoid arthritis was reported in 1928 in four generations of women in one family by Kroner (Lawrence, 1970). Twin studies have demonstrated a genetic component to RA disease susceptibility. Hospital treated identical twin concordance rates and sibling recurrence risks are both higher than those in community based studies. A hospital based ARC twin study (Lawrence, 1970) examined 38 identical twins and found a rate of 30% for concordance in seropositive erosive RA. This contrasts sharply with the findings of a community based population study in Finland (Aho et al, 1986) of only a 12% concordance rate. The differences between these two studies may be explained by community based RA being less familial than hospital treated RA (Deighton CM, 1989). Secondly the ARC study only studied seropositive erosive RA, as opposed to the Finnish study which included both seronegative and seropositive cases. If the seronegative twins in the ARC study are included the concordance rate falls to 16%, a similar figure to the Finnish study. This suggests that familial/genetic factors are not so important for the initiation of the rheumatoid process but

play a more important role in the development of disease severity and hence the stronger association between seropositive erosive and increased twin concordance rates.

Studies have attempted to establish the extent of the familial nature of RA by recording the proportion of RA patients with a first-degree relative (usually a sibling) with RA. A number of these studies have demonstrated a small increase in risk to first-degree relatives. These studies (Schull WJ, 1969;Veenhof-Garmann, 1968;Bennett PH, 1968 and Hellgren L, 1970) were undertaken before there was a more stringent definition of RA (ARA criteria, Arnett FC et al, 1988). More recent community based studies have shown that the risk of RA among first-degree relatives is only 1.6-1.7-fold higher than in the general population (Jones MA, 1996 and del-Junco D, 1994). However, a hospital based study observed an increased risk of hospital based RA if a family member also has the disease, odds ratio (OR) = 6. (Koumantaki Y et al, 1997).

Previous studies on the differences between familial and sporadic RA have reported conflicting results. Some studies have found no difference at all, whilst others have found phenotypic differences in gender, age at disease onset and sibship size.

None of these studies have addressed the smoking history as a possible factor that differs between sporadic and familial RA cases. For example a study by Radstake et al, 2000 observed that males, but not females with familial RA were significantly younger at disease onset than those with sporadic RA (median 50 yrs vs. 57 yrs. P=0.03). The possible reasons for this striking difference were not discussed. In section 1.1.c of this thesis (Literature review of smoking and RA susceptibility) it is apparent that smoking is an important environmental factor in RA development. The striking difference in cigarette smoking between continental European female (40% ever smoked, Hazes et al, 1990) and male RA patients (90% ever smoked, Uhlig T, 1999) may explain the differences observed by Radstake et al, 2000. Therefore if an individual smokes and has a family history of RA

(potential additive effect of two susceptibility factors) the age of presentation may be significantly earlier than an individual who smokes and has no family history of disease. Whereas comparing individuals who have never smoked with or without a family history of RA may not reveal such a striking difference. No studies have examined the relationship between cigarette smoking and a family history of RA.

1.1.c Literature review of smoking and RA susceptibility

There is an increasing literature regarding smoking and RA susceptibility. There have been reviews or editorials on this subject (Silman, 2002;Symmons, 2002;Albano, 2001; Shovman, 2000;Wilson, 1999;Deighton, 1997; Silman, 1993).

Earlier epidemiological studies, however, did not consider smoking as a candidate for RA susceptibility (Lewis-Fanning E, 1950). In fact the possibility of an association between smoking and RA was apparent in 1967 in a study of the relationship between lung disease and RA (Walker WC, 1967). This study of hospital based RA patients from the North of England observed a significantly increased prevalence of smoking in RA patients compared to control cases. Of the RA men studied only 7% had never smoked and the principal difference between the male RA cases and their respective controls was in those smoking 11 to 20 cigarettes daily for more than 20 years. Interestingly analysis of those women smoking for 11 years or greater reveals a significantly higher proportion of RA female cases than control cases (32% vs. 23%) smoking for this duration.

Further studies investigating a possible link between RA and the oral contraceptive showed evidence of an increased risk of RA in smokers. The Oxford Family Planning Clinics survey (Vessey et al, 1987) observed a relative risk of 2.4 for those smoking over 15 cigarettes per day. In the Nurses Health study (Hernandez-Avila, 1990) the relative risk in current smokers was small (1.3) and non-significant. Hazes et al, 1990 are the only group to have reported a protective effect of smoking on RA development. In this Dutch retrospective study current smoking was associated with a risk of RA only 60% of that of non-smokers. This study was of female pre-menopausal RA cases only. This is an important study as it highlights that there may be considerable differences between earlier onset and older onset RA patients and the gender of RA patients in terms of their smoking histories. It could be argued that if there is a dose dependant mechanism involved between cigarette smoking and

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RA susceptibility those RA patients with early onset disease would not have had time to accrue the number of years smoked to be at risk of RA. Other factors, therefore, such as genetic factors are likely to be important in the triggering of RA in those RA cases with early onset disease.

The first study to specifically investigate the relationship between cigarette smoking and RA (Heliovaara et al, 1993) of a substantive population based prospective study in Finland observed that smoking is a risk factor for seropositive RA in men but not in women. The relative risk of seropositive RA was 2.6 (95% CI, 1.3-5.3) in male ex-smokers and 3.8 (95% CI, 2.0-6.9) in current smokers, in comparison with the men who had never smoked. Age, geographical location or social class did not confound these findings. Again it was noted that only a small minority (10%) of the male seropositive cases were lifelong non-smokers (compared to 30% in the general population). There are a number of important points to be made from this study. Firstly there was no association between cigarette smoking and seronegative RA, but only seropositive RA. This firstly raises the possibility that cigarette smoking induces RA susceptibility via the production of RF and secondly that cigarette smoking is not associated with the rheumatoid process per se. Studies, therefore, concentrating on community based RA patients where there is a strong predominance of seronegative RA cases may not observe an increased frequency of smoking in such patients. Again this study highlighted the importance of quantifying the duration of smoking as a twenty fold increased incidence of RA developing in healthy men currently smoking as opposed to healthy men who had never smoked after individual follow up of 14 or more years was observed. This suggests a possible latent affect of cigarette smoking. This affect may continue despite smoking cessation and may explain the findings that ex-smokers are at an increased risk of RA. Finally, the prevalence of smoking in the female population studied was very low with almost 80% of the women never having smoked and only 2% currently

smoking greater than 15 cigarettes per day. This raises the real possibility that if smoking is associated with prolonged heavy smoking that very few of the control women studied were exposed to this risk factor.

Voigt et al, 1994 conducted a population-based case-control study of RA in North America. 349 incident cases of RA were compared with 1,457 controls. Women with 20 or more pack-years of smoking had a relative risk of 1.5 (95% CI = 1.0-2.0) compared with never-smokers. Following this study Silman, 1996 observed that cigarette smoking increased the risk for RA. Discordant twins were studied and it was found that there was an increased risk of developing RA in identical twins who had ever smoked OR=12 (95% CI 1.78-513). This risk was similar for female identical twins OR=10 (95% CI 1.4-434).

A British community based study (Symmons et al, 1997) observed that individuals who had ever smoked had a higher risk of developing seropositive RA OR 2.9, (95% CI 0.92-9.21) than seronegative RA OR 1.2, (95% CI 0.7-2.09). They observed no increased risk for current cigarette smoking and RA. A larger North American community based study of female health professionals (Karlson et al, 1999) had sufficient power to show smoking to be a slight, but significantly increased risk of RA. The OR for the onset of seropositive RA increased along with the magnitude of cigarette consumption from 1.1 (CI 1.04-1.19) for 0-14 cigarettes smoked per day to 1.4 (CI 1.28-1.58) for over 25 cigarettes per day. However, smoking intensity (number of cigarettes/day) was unrelated to risk of seropositive RA, after adjustment was made for duration of smoking. A large Norwegian study (Uhlig et al, 1999) investigated 361 RA patients and almost 6000 randomly selected individuals. Again current smoking in men with seropositive RA was a risk factor, OR 4.8 (95% CI 2.09-10.9), but not among those who were seronegative, OR 1.57 (95% CI 0.8-3.06). For women there was no significant association between current smoking and seropositive, seronegative, or overall RA. These findings were independent of employment status and education level.

A Swedish study (Olsson et al, 2001) investigating co-morbidity and lifestyle in hospital outpatients based RA observed current and previous smoking were associated with increased risks for seropositive RA in both sexes. In both sexes there was a dose-response relationship found with number of tobacco pack years (p for trend <0.005 men and p=0.029 women.). The number of pack years smoked were banded <5, 5-9, 10-19, >20. Therefore high intensity smoking and modest smoking were grouped together. Current and previous smoking were associated with increased risks for RA in both sexes, and in men a dose-response relationship was found with number of tobacco pack years (p for trend <0.005). For men smoking > 20 pack years the risk for seropositive RA was increased significantly OR 3.4 (1.5-8.4) and for women OR 2.5 (0.9-6.7).

Criswell LA, 2002 studied whether cigarette smoking increases the risk of RA among postmenopausal women. A cohort of 31 336 women in Iowa who were aged 55 to 69 years in 1986 and who had no history of RA. Through 1997, 158 cases of RA were identified. Multivariable Cox proportional hazards regression was used to derive rate ratios (RRs) and 95% CIs for the association between cigarette smoking and RA. Compared with women who had never smoked, women who were current smokers (RR = 2.0; 95% CI: 1.3 to 2.9) were at increased risk of RA. Those who had quit 10 years or less before study baseline (RR = 1.8; 95% CI: 1.1 to 3.1) were at increased risk of RA, but women who had quit more than 10 years before baseline were not at increased risk (RR = 0.9; 95% CI: 0.5 to 2.6). Both the duration and intensity of smoking were associated with RA. Multivariable adjustments for age, marital status, occupation, body mass index, age at menopause, oral contraceptive use, hormone replacement therapy, alcohol use, and coffee consumption did not alter these results.

Additionally Masi, 2001 published his investigations regarding the relationship between RA and heavy cigarette smoking. A case-control study nested within a community based cohort (n=21061 adults) enrolled in 1974. Twenty years later 54 individuals were

noted to develop RA. These were matched with four controls from the entry cohort and were matched for age, sex, and race (all white subjects). Heavy cigarette smoking (>30 cigarettes per day) was associated with RA (OR 21.5, 95% CI 1.9 to 122.5, p=0.005). A very recent Finnish study (Krishnan E, 2003) studied 1095 patients with RA and 1530 control individuals. Preliminary analyses revealed the presence of substantial statistical interaction between smoking and sex (P < 0.001). In separate multivariable analyses, past history of smoking was associated with increased risk for RA overall in men (odds ratio 2.0, 95% confidence interval 1.2-3.2) but not in women. Among men, this effect was seen only for seropositive RA.

Taking all previous studies together there is clearly a relationship between cigarette smoking and the development of RA. All of these studies investigated the prevalence of smoking at disease onset and therefore the issue of RA itself influencing cigarette consumption is irrelevant. An important feature would appear to be the duration of smoking and possibly the intensity of smoking. There is a relationship between smoking and seropositive as opposed to seronegative RA raising important questions as to the pathogenesis of RA.

1.1.d Literature review of smoking and RA severity

The relationship between RF and smoking in RA patients is discussed in section 1.2a. There would appear to be a consensus of opinion that smoking in RA is associated with RF production. However regarding the relationship between cigarette smoking and disease severity measured in terms of joint erosion, the number of swollen joints, the need for joint replacement and the level of functional disability is still a matter for debate. This subject has been reviewed by Harrison BJ, 2000 and 2002.

There is agreement however that rheumatoid nodules and rheumatoid vasculitis are associated with cigarette smoking. Rheumatoid vasculitis is a life threatening complication of RA and patients with this complication have more severe RA with destructive joint disease, rheumatoid nodules, and high titres of rheumatoid factor (Scott DGI, 1981). Three studies have demonstrated an association between current cigarette smoking and rheumatoid vasculitis (Struthers, 1981; Voskuyl, 1986; Harrison, 2001).

Rheumatoid nodules were first observed to be associated with cigarette smoking in a North American study (Saag, 1997). The presence of subcutaneous rheumatoid nodules was associated with pack years of smoking, although this failed to reach significance and no dose relation was found. Current smoking was not significantly associated with rheumatoid nodules.

Further work by Wolf, 2000 observed with logistic regression analyses, current or previous smoking was associated with an increased risk for the presence of rheumatoid nodules: OR 2.35 (1.6,3.46), even after controlling for RF positivity. Similar results were obtained when analysed by years of smoking. Masdottir, 2000 observed that RA individuals smoking 20 pack years or more to be significantly more likely to have rheumatoid nodules than those who had smoked less than 20 pack years (45% vs.16%). A study of early inflammatory polyarthritis (Harrison, 2001) observed that nodular RA was significantly more

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frequent in those smoking for 35 years or more compared to individuals who had never smoked OR 4.2 (95% CI 1.31-13.6). In contrast to this, those individuals who had smoked for 0-18 years did not have an increased prevalence of nodular disease compared to those who had never smoked OR 0.78 (95% CI 0.18-3.18).

Finally Mattey 2002 observed a significant association between current cigarette smoking and rheumatoid nodules. Current smokers were more likely to have nodular disease than those who had never smoked (OR 1.8, 95% CI 1.0-2.9). An association was also found between RF positivity and nodular disease (OR 2.2, 95% CI 1.2-3.8) that remained significant after correction for current smoking. A combination of current smoking and seropositivity increased the risk of nodular disease (OR 3.9, 95% CI 1.7-9.1). There was no significant association between ex-smokers and rheumatoid nodules.

Rheumatoid erosions are an important end-point in RA and radiographically detected juxta-articular bone erosions are a characteristic consequence of longstanding, severe RA and provide a cumulative index of joint damage (Van der Heijde, 1988 and Sharp, 1989). Saag et al, 1997 observed pack years of cigarette smoking to be associated with radiographic erosions. After adjustment for potential confounders smokers with 25 or greater pack years were 2.4 times more likely to exhibit radiographic erosions (95% CI 1.2,4.5) than never smokers. Smoking was also positively associated with the Larsen score, a quantitative measure of RA radiographic damage. However, moderately severe radiographic disease seemed to be more strongly associated with cigarette smoking than more severe disease. When RF was placed into the same statistical model with smoking, the smoking and radiographic erosion association was no longer statistically significant. Finally there was no association between current smoking and the presence of radiographic erosions

Wolfe, 2000 extended the work regarding rheumatoid erosions and cigarette smoking in a hospital based study. Smoking was observed to be associated with rheumatoid erosions.

This effect was not detected in linear models or when comparing smokers versus non-smokers. However, when non-linear methods were used effect of smoking on erosions could be seen in patients with very long smoking histories. There is virtually no effect even after 40 years of smoking, but a non-linear increase thereafter. This finding was found to be independent of RF.

Masdottir, 2000 observed that smoking was significantly associated with more radiological joint damage and was more marked in those smoking 20 pack years or more and also significantly worse in those smoking at diagnosis compared to those not smoking at diagnosis.

Contrary to these findings Harrison, 2001 observed that smoking status had no influence on the development of erosions in patients with early inflammatory polyarthritis 3 years after the onset of their disease. This study did not quantify the number of cigarettes smoked in terms of pack years. However, no association was found between the number of years smoked and the presence of erosions or the Larsen score.

An important endpoint in RA is whether the patient requires joint replacement. The need for multiple joint replacement is perceived to be associated with more severe and progressive RA. A study investigating clinical parameters associated with rheumatoid joint replacement (Wolfe, 1998) observed that smokers were significantly less likely to require joint replacement. However this study also observed that the presence of RF did not predict joint replacement, which is surprising when one considers that RF is an independent and powerful predictor of progressive joint damage. In this study approximately one third of the joint replacements were of the knee and it is possible that RA smokers tend not to have large joint involvement or that a number of the joint replacements are as a result of secondary osteoarthritis. Interestingly cigarette smoking is protective for the development of

osteoarthritis (Felson, 1989; Felson, 1997; Samanta, 1993) and this in part may explain the findings of Wolfe, 1998.

Other clinical parameters frequently measured in RA include the number of inflamed joints, functional disability as measured by the Health Assessment Questionnaire (HAQ) and the acute phase response as measured by the ESR and CRP. Both Saag, 1997 and Wolfe, 2000 did not observe significant univariate relations between smoking and the above measures of disease activity or severity. In contrast (Masdottir, 2000) observed a significantly higher HAQ score in individuals smoking more than 20 pack years, but no significant increase in the number of swollen joints. Harrison, 2001 observed no association between current smoking and the HAQ score and in fact observed significantly fewer swollen joints in the current smokers (OR 0.61, 95% CI 0.37-0.98).

1.1.e Social class and RA

It is important to consider socio-economic class when one is addressing the impact of cigarette smoking upon RA as discussed in section 1 there is a strong relationship between the prevalence of cigarette smoking and social class in Britain.

The effect of socio-economic disadvantage on the outcome of RA is now well documented. The initial studies investigating the relationship between social deprivation and RA severity used formal education as a surrogate marker of socio-economic status. In these studies, Callahan LF, 1988 and 1994 demonstrated that outcome of RA in terms of laboratory and functional measurements of disease activity is inversely related to the number of years of formal education in the USA. These findings were reproduced by Leigh JP, 1991 and further reports from the USA (Mitchell JM, 1988) and the Netherlands (Vliet Vlieland TPM, 1994) have suggested that outcome in RA is worse in patients of lower socio-economic status.

In Britain, two large studies have demonstrated a clear link between socio-economic deprivation and a poor outcome of RA. In a study of 814 Glaswegian RA patients (McEntegart, 1997) patients from deprived areas were found to have significantly poorer function at both disease onset and at five years. Those in the Carstairs deprivation category 1 and 2 (least deprived) had a median HAQ score of 1.12 (0-2.75) compared to those in category 6 and 7 (most deprived) with a significantly higher HAQ score of 1.88 (0-3). This finding could not be attributed to differences in disease duration or compliance with treatment. A large prospective cohort of patients enrolled in the Early Rheumatoid Arthritis Study (ERAS Study Group, 2000) studied 869 consecutive patients with RA from nine hospital rheumatology clinics. Socio-economic deprivation was significantly associated with a worse clinical course (HAQ score, joint score, grip strength, functional grade) and this effect was already apparent at presentation.

A number of reports have investigated the link between lower socio-economic class and RA susceptibility. A North American survey found a lower disease frequency in males in the highest social groups (Engel A, 1968) whereas a British survey suggested a lower frequency in females in the lowest social groups (Lawrence JS, 1977). A NOAR study (Bankhead C, 1996) observed that RA is not related to indicators of socio-economic deprivation. However these surveys were based on a relatively few ascertained cases and did not discriminate between seropositive and seronegative RA cases. For example the NOAR study only categorised 49 men and 102 women into five social groups. This group of patients were community based and therefore unlikely to be representative of more severe hospital treated RA cases. Additionally no adjustment was made for the fact that individuals of lower socio-economic status die earlier than individuals of high socio-economic status and that the incidence of RA increases markedly with increasing age. This study did however find that the incidence of RA was highest for both males and females in those currently employed as skilled manual workers.

1.2 Rheumatoid factor and RA

Waaler's observation of high levels of serum RF in patients with RA radically changed the earlier concepts of RA as a disease purely of abnormal connective tissue metabolism. Presence of IgM RF in RA is the sole serologic indicator included in the ARA disease criteria (Arnett et al, 1988). Rheumatoid factor is detected in 75-80% of hospital treated RA patients. Rheumatoid factor is a consistent predictor of joint severity in RA (Bukhari M, 2002). Bukhari identified 12 studies that demonstrated that RF was associated with worsening of rheumatoid joint disease. Rheumatoid factor is also found in other conditions and these are listed in table 1.

Rheumatoid factors are antibodies directed against gammaglobulins. These autoantibodies appear to be synthesised in response to immunoglobulins that have been conformationally changed. The most common RF is an IgM antibody to IgG. Polyclonal IgM of the sera of patients with RA react with a diverse array of antigenic determinants localised to the Fc portion of the IgG molecule in both the CH2 and CH3 domains (Johnson et al 1976). Polyclonal IgM RF also reacts with neoantigens better expressed on aggregated, denatured, or enzymatically digested IgG than on monomeric IgG. (Henney, 1966; Metzger H, 1974). In spite of its intrinsically low affinity for antigen, RF can produce stable complexes with IgG particularly when the IgG antigen is aggregated (Metzger H, 1974). The synovial fluid of RA patients unlike the serum frequently has markedly depressed complement levels and contains high molecular weight IgG aggregates (Hannestad K, 1967; Winchester, 1970). IgM RF can fix complement (Tanimoto 1975). This process is important in RA and induces inflammation of the synovium. Rheumatoid factors in RA, when compared to non-rheumatic cases, are of higher titre, react better with animal gammaglobulins, are produced primarily in the synovium and other extravascular sites. There are a number of mechanisms by which RF may be formed in RA. Aggregation of IgG

in the rheumatoid joint may potentially lead to enhanced binding of IgG to low affinity receptors on potential RF forming cells (Metzger H, 1974; Eisenberg R, 1976). The creation of neoantigens on the Fc portion of IgG by the combination of antibody with antigen, immunoglobulin aggregation, or denaturation (Henney CS, 1966) or the ability of exogenous antigen trapped in an immune complex to stimulate helper T lymphocytes, and hence to break self tolerance (Weigle WO, 1973). Previous studies have suggested that free radical mediated alteration of IgG may stimulate the formation of immune complexes with RF antibody, thereby promoting tissue damage during rheumatoid inflammation (Lunec J, 1988;Swaak AJ, 1989).

Lastly lymphocytes from normal individuals secrete low affinity IgM RF after mitogenic stimulation by polyclonal B cell activators including pokeweed antigen (Dresser DW, 1978) and Ebstein Barr virus (Slaughter L, 1978). Ebstein Barr virus is a permanent but quiescent resident of the B lymphocyte and can be reactivated by autoantibodies (Tovey M, 1978) raising the possibility of feedback mechanism. Rheumatoid arthritis patients who share certain HLA-D related antigens are more frequently seropositive than those who lack these antigens (McMichael, 1977). However in normal individuals IgM RF was not associated with a particular HLA type (Engleman, 1978).

Sustained RF production may depend upon the continual presence of immunological stimuli. A good example of this is the disease sub-acute bacterial endocarditis (a chronic infection of the heart valve). This disease is associated with immune complex formation and RF is frequently present, elimination of bacteria by antibiotics leads to the subsequent decline in RF. (Carson DA 1978).

Table 1. Diseases commonly associated with rheumatoid factor

1. **Rheumatoid arthritis**	
2. Other rheumatic diseases	Systemic Lupus Erythematosus, systemic sclerosis, Sjogrens syndrome , mixed connective tissue disease.
3. Acute viral infections	Mononucleosis (glandular fever), hepatitis, influenza and following some vaccinations.
4. Parasitic infections	Trypanosomiasis, malaria and schistosomiasis.
5.Chronic inflammatory conditions and bacterial infections	Tuberculosis, leprosy, syphilis subacute bacterial endocarditis and bronchiectasis
6. Neoplasms	Particularly following irradiation or chemotherapy
7. Hyperglobulinaemic states	Hyperglobulinaemic purpura, sarcoidosis, chronic liver disease, cryoglobulinaemia, Waldenstroms macroglobulinaemia.

1.2.a Smoking and rheumatoid factor in RA

The first study to specifically investigate the relationship between cigarette smoking and seropositive RA (Heliovaara et al, 1993) of a substantive population based prospective study in Finland observed that smoking is a risk factor for seropositive RA in men but not in women. The relative risk of seropositive RA was 2.6 (95% CI, 1.3-5.3) in male ex-smokers and 3.8 (95% CI, 2.0-6.9) in current smokers, in comparison with the men who had never smoked. There was no significant association between smoking and seronegative RA.

Saag et al, 1997 demonstrated a strong association between smoking and the presence of RF in individuals with RA. This was particularly striking for those smoking more than 25 pack years. These individuals were 3 times more likely to be RF positive (latex fixation RF) than RA individuals who had never smoked. This relationship was not confounded by socio-demographic variables. Following this important study a number of studies have reported similar findings.

Houssien et al, 1998 observed that active RA smokers had significantly higher IgA RF levels compared to non-smoking RA patients. A high proportion of the RA smokers (67%) were both IgA and IgM RF positive compared to only 26% of non-smoking RA patients.

Wolfe, 2000 studied a large cohort of 610 RA patients and found RF concentration was linearly related to the number of years smoked and that this association was stronger in men. For forty years of smoking in RA men the median RF levels were three times higher than male RA non-smokers. Interestingly current smoking did not differ from past or all smoking in its effect on RF.

Masdottir et al, 2000 observed a significant association between the number of pack years smoked and the levels of IgA and IgM RF, but not IgG RF in RA patients. A recent community based study of early polyarthritis (Harrison et al, 2001) observed that current

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smokers were significantly more likely to be RF positive at diagnosis (47%) than were ex-smokers (34%) and never smokers (31%).

Clearly these large studies suggest in RA patients that there is a relationship between cigarette smoking, duration of cigarette smoking and RF levels.

1.2.b Smoking and rheumatoid factor in healthy individuals

Mathews et al, 1973 were the first to observe a relationship between cigarette smoking and the development of autoantibodies in healthy individuals. Their study observed a relationship between cigarette smoking and antinuclear antibodies in males and females of all ages from Western Australia. Considering the strongest ANA reactions only, the relative risk (smokers v. non-smokers) was increased to approximately 9 for men and 4 for women. Furthermore, there was an effect of quantity of cigarettes smoked per day with respect to ANA reactions. The relationship between smoking status and RF was found to be age dependent, with an excess of RF in young male smokers (21-40), relative risk approximately 2. The RF concentration was not quantified in this study and a potential relationship between smoking intensity and RF was not investigated.

Lawrence JS, 1971 in a study of the industrial North of England observed the prevalence of a false positive RF reaction was higher in polluted areas than less polluted areas, although no account for cigarette smoking was made. A further larger community based study based in Finland (Tuomi 1990) observed a relationship between a positive RF reaction and cigarette smoking in healthy individuals. Rheumatoid factor testing was performed by the sensitised sheep cell agglutination test. 'False positive' RF reactions occurred twice as often in both male and female current smokers and ex-smokers than in those who had never smoked. The prevalence of high titres of RF (>500) was fourfold greater among current smokers than among those who had never smoked. These associations were statistically significant and independent of age and lung function.

The relationship between smoking and serum levels of individual RF isotypes was studied (Jonsson, 1998) in elderly Icelanders born between 1907 and 1935. This study observed smoking to be most prevalent (45%) amongst participants with elevation of both IgM and IgA RF and that smokers were also significantly more likely to have a persistent

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elevation of RF than non-smokers. Of the RF-negative participants, 22% were active smokers compared to significantly more of the IgM RF and IgA RF-positive individuals (34%). Interestingly there was no relationship between smoking and IgG RF. Smoking was similar between the RF positive men and the RF positive women.

A recent study investigating the relationship between coffee consumption and RF in healthy individuals (Heliovaara et al, 2000) observed that cigarette smoking was the most important known environmental factor for the development of a positive RF in the general population. Interestingly lower socio-economic status was not associated with RF production when adjustment was made for cigarette consumption. Smokers when compared to individuals who had never smoked were 4 times more likely to have a positive RF (sensitised sheep cell agglutination titre >128) adjusted for age, sex and coffee consumption. Furthermore former smokers when compared to individuals who had never smoked were almost 3 times more likely to be RF positive. Increasing age was also associated with a positive RF and was independent of smoking. In further work in this area Korpilahde T, 2003 observed that there was a close association between smoking and strongly positive RF (titre >128), and a weaker association between smoking and RF with a lower titre (>32). Adjusted for sex, age and coffee consumption, the odds ratio (95% confidence intervals) of strongly positive RF in those who smoked >20 cigarettes a day was 3.46 (1.43-8.35). In smokers of less than 20 per day the risk was 4.12 (2.08-8.17) and in former smokers the risk was 2.67 (1.31-5.47) compared with those who had never smoked. Age was observed to be an independent risk factor of strongly positive RF. For age 30-44 OR 1.00, 45-54 OR 1.96 (1.03-3.73), 55-64 OR 1.6 (0.76-3.36), 65-75 OR 2.36 (1.1-5.01), over 75 OR 4 (1.61-10.15). Therefore the concept of pack years smoked is likely to be of importance regarding the relationship between smoking and RF production in healthy individuals. A pack year is equivalent to smoking twenty cigarettes per day every day for one year. It is therefore a

measure of both smoking intensity and the duration of exposure. Pack years have long been utilised by cardiology and respiratory physicians to assess an individual's risk for developing diseases such as lung cancer, myocardial infarction and pulmonary emphysema. As explained earlier there is a relationship between pack years smoked and RF positivity in RA patients and a relationship between pack years smoked and RF concentration. As positive RF reactions commonly precede the onset of clinically manifest RA, it is an important concept to assess susceptibility to developing RA as a result of cigarette smoking in terms of pack years smoked. Almost all the studies investigating the relationship between smoking and RA, however, have failed to quantify the number of cigarettes smoked.

1.3 The influence of rheumatoid factor and RA development

A number of studies have addressed the question as to whether the production of RF in healthy individuals precedes the development of RA. All of these studies suggest a relationship between RF production in healthy individuals and the development of RA. The first study to address the relationship between a positive RF reaction and the development of RA (del Puente A, 1988) observed a striking association between increasing RF titre in healthy North American Indians and the development of RA. During the study period, 70 new cases of RA developed. When the population at risk was stratified by RF titre, the age- and sex-adjusted incidence of RA increased with higher titres of RF. The incidence of RA (in cases per 1,000 person-years) according to RF titre was 2.4 (RF titre less than 1:2); 6.7 (titre 1:2-1:16); 11.0 (titre 1:32-1:256); and 48.3 (titre greater than 1:256) (P less than 0.001). The same trend was also found within each age and sex group, and within groups defined by the number of American Rheumatism Association criteria present before the definite diagnosis. Del Puente concluded that the presence of RF, in subjects without RA, is a risk factor for the development of RA, and that this risk is related to the RF titre.

Population studies have shown that the majority of people with increased RF have no symptoms of rheumatic diseases or chronic infections (Jonsson T et al, 1992).

Aho et al, 1985 and 1991 observed that a positive RF in healthy individuals preceded the development of seropositive RA. These findings were confirmed in a further study (Walker DJ et al, 1986). A recent prospective study on the incidence of RA among people with persistent increase of RF (Halldorsdottir HD et al, 2000) compared the incidence of developing RA in healthy people with transient or a persistent increase of one or more RF isotypes. They observed that healthy individuals with a combined increase of IgM and IgG RF had a relatively stable RF, with 85% of these individuals remaining RF positive over a mean of 16.5 years follow up. Those individuals with two or more RF isotypes positive at

baseline were noted to have an annual incidence of developing seropositive RA 23 fold

higher than expected. Those individuals with one RF isotype positive had a 7 fold increased

annual incidence of developing RA. These data suggests that symptom free persons with

persistently raised RF have a greatly increased risk of developing RA and suggests that

dysregulation of RF production is a predisposing factor in RA.

1.4 Neutrophil elastase and RA

Neutrophil elastase (NE) has been extensively studied with respect to its role in the development of pulmonary emphysema (Shapiro SD, 2002). Neutrophil elastase is produced during neutrophil differentiation, and is packaged and stored in the azurophil granules prior to release of the cells from the bone marrow. Neutrophil elastase is a serine proteinase (serine at the active site) with a broad range of substrate specificities. Firstly it can degrade elastin and other connective tissues including types III and IV collagens, proteoglycans and fibronectin (Janoff A, 1985). It can also degrade immunoglobulins (Niederman MS, 1986) and the C3b1 receptor on neutrophils that is responsible for phagocytosis and bacterial killing. It also increases IL8 production by bronchial epithelial cells and since this cytokine is also a potent neutrophil chemoattractant there is a potential initiation of a self -perpetuating process (Tanino, 2002).

The MMP inhibitor TIMP 1, is an important inhibitor of the specific MMPs that cause rheumatoid joint damage (Kolkenbrock H, 1991). Neutrophil elastase has been demonstrated to inhibit TIMP 1 (Nagase H, 1997). Furthermore, NE activates latent stromelysin-1 (Nagase H, 1997), an important MMP involved in joint destruction (Van Meurs J, 1999).

Neutrophil elastase has been proposed to account for at least some of the tissue damage occurring in RA and experimentally it has been demonstrated that NE can degrade articular cartilage (Velvart M, 1981, Schalkwijk J, 1987, Janusz MJ, 1991, Moore AR, 1999).

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1.5 Alpha 1- proteinase inhibitor

Western Europeans are particularly prone to disease arising from a genetic deficiency of the plasma protein $\alpha 1$-PI. Alpha 1- proteinase inhibitor is the archetype of the serine proteinase inhibitor. Members of the serine proteinase inhibitors have closely related structures and functions. These inhibitors control the various inflammatory cascades including coagulation (antithrombin), complement activation (C1-inhibitor) and fibrinolysis ($\alpha 2$-antiplasmin) and angiotensinogen with a similar structure to the rest of the family but without proteinase inhibitory capacity.

Alpha 1- proteinase inhibitor is subject to genetic variation resulting from mutations in the 12.2 kb, 7-exon gene at q31-31.2 on chromosome 14. Over 75 allelic variants have been reported and classified using the Pi (proteinase inhibitor) nomenclature, which assesses $\alpha 1$-PI mobility in isoelectric focusing analysis. Mutations are inherited by simple Mendelian trait. The most common variant is the M allele (subdivided into M_1, M_2, M_3, M_4, M_5) which codes for about 1.4g/l of the protein in plasma. Approximately 85% of the population have the MM phenotype and therefore the normal concentration of $\alpha 1$-PI is 2.8 g/l.

Human $\alpha 1$-PI is a single glycosylated polypeptide chain of 394 amino acid residues with a molecular mass of 52KDa. $\alpha 1$-PI inhibits several members of the serine proteinase family. Trypsin is included among the proteinases inhibited by $\alpha 1$-PI, hence an earlier term of $\alpha 1$-antitrypsin. The protein is synthesized and secreted predominantly by hepatocytes, which determine the circulating levels of $\alpha 1$-PI and hence tissue levels. Some synthesis and secretion of alpha 1-PI can occur in the lung as a result of macrophage and neutrophil production and secretion.

The principal physiological function of $\alpha 1$-PI is the inhibition of NE. This inhibitory process occurs as a result of an irreversible 1:1 complex between $\alpha 1$-PI and NE. This

complex formation is dependent on the reactive site of α1-PI that lies on an exposed, loop structure (reviewed by Hiemstra PS, 2002). The scissile peptide bond is Met[358]-Ser[359] and the Met residue side chain fits well into the reactive site of NE, thus providing specificity for the interaction. Oxidative damage to Met[358] results in loss of structural integrity and a marked reduction in the association rate constant (2000 fold decrease) between α1-PI and NE (Taggart C, 2000). Janoff A, 1986 demonstrated that exposure of α1-PI to oxidants such as hypochlorous acid, hydrogen peroxide or N-chloramines results in the oxidation of Met[358]. The position of Met[358] on a loop structure readily exposed to the oxidising agent explains the relative ease by which Met[358] is oxidised. Exposure of α1-PI to oxidants causes loss of α1-PI activity, which is expressed as elastase inhibitory capacity (EIC).

1.5.a Alpha 1- proteinase inhibitor and RA

Before reviewing the relatively few papers investigating the link between RA and the α1-PI allele it is important to consider a number of important points. Firstly the Pi ZZ phenotype is extremely rare, only 0.03% (30/100000) individuals in the UK are Pi ZZ phenotype (Cook PJL, 1975). Genetic abnormalities are often reported with modest associations with RA (OR 2-3). However a comparable risk regarding the α1-PI ZZ phenotype would result in less than 0.1% of the study population having the Pi ZZ phenotype. Therefore the study size required to demonstrate a risk for Pi ZZ individuals would need to be extremely large.

Secondly RA is an extremely heterogeneous disease and it is possible that individuals with α1-PI deficiency are more likely to be of a certain phenotype. Rheumatoid factor has been demonstrated to cause neutrophil degranulation with the subsequent release of neutrophil elastase (Shingu M, 1987). A deficiency of α1-PI may therefore act synergistically with RF to up regulate neutrophil elastase activity and predispose an individual to erosive disease. Therefore the Pi Z mutant allele may be associated with more severe forms of RA rather than the persistently non-erosive milder type of RA.

Thirdly, there is a potential interaction between cigarette smoking and the Pi SZ, Pi ZZ, phenotypes as is the case for pulmonary emphysema. The phenotype Pi MZ is not thought to be a susceptibility factor in the development of emphysema without a history of cigarette smoking (Walter R, 2000). Therefore, the risk of developing RA may be only increased in specific Pi phenotypes such as Pi ZZ and Pi SZ as is broadly the case with pulmonary emphysema.

In total there have been 9 studies investigating the relationship between α1-PI and RA. There is controversy about the prevalence of α1-PI deficiency alleles in adult patients

with RA. Unfortunately no consensus emerged from these studies and the role of Pi phenotypes has not been studied since 1989.

The first study in 1976, by Cox and Huber suggested that there was a significantly increased prevalence of Pi Z heterozygotes, as they found a prevalence rate of 9% in 55 patients. This study was prompted by the anecdotal observation that Pi ZZ individuals had a relatively high prevalence of severe deforming RA. For example of 24 patients reported (Laurell and Eriksson, 1963) in which a case history was given and were 45 years or older, one had RA. In a series of Pi ZZ patients investigated by Cox and Huber (unpublished data) of 21 adults over the age of 45, one of these patients had severe disabling RA from sixty years of age. The preliminary report by Cox and Huber sparked great interest and controversy over the subsequent 12 years.

Sjoblom et al, 1976 reported a larger study of 255 Swedish seropositive RA patients and 65 seronegative RA patients. They found no significant over representation of Pi MZ or MS individuals in their patients. However, the prevalence of the Pi MZ phenotype reported was 5%, far higher than the reported prevalence of Pi MZ (1.5%) in 4565 healthy British individuals (Cook PJL, 1975). Collins et al, 1976 did not observe an increased frequency of Pi MZ in RA, but some less common Pi types also associated with low $\alpha 1$-Pi levels were increased. Brackertz et al, 1977 studied 95 Swiss RA patients and found no association with the Pi MS and MZ phenotypes. Similarly no association was observed in English RA patients (Geddes et al, 1977), however they reported an interesting association between non-Pi MM phenotypes and fibrosing alveolitis. They found the risk of fibrosing alveolitis in individuals with non- Pi MM phenotypes to be increased 6 times and this increased to almost 10 times in the presence of RA.

In 1980, Cox and Huber reported an increased prevalence rate of 9% of Z heterozygotes in 108 patients with classical RA. Their study suggested that the Z

heterozygotes had more severe disease, as 9 of their 10 heterozygotes had severe functional disease (stage III or IV disease). They suggested further that these Pi Z heterozygotes were significantly more likely to be women with a younger age of disease onset.

Further work in Sweden (Beckman et al, 1984) of 200 RA patients revealed a significant association between Pi Z heterozygotes and RA. It is noteworthy that the patients studied were a well-defined RA population. All the patients studied were erosive and 97% were seropositive. There was a significantly increased prevalence of Pi heterozygotes in the male but not the female patients. Smaller studies investigating the α1-PI phenotype of RA patients with or without pulmonary fibrosis observed an increased prevalence of the Pi ZZ phenotype (Michalski et al, 1986) and Pi MZ phenotype (10%), (Hietala et al, 1987) in RA patients. α1-PI phenotypes were defined in 144 RA patients (Sanders et al, 1986) and no increase in frequencies of the rare α1-PI phenotypes were observed.

A large study of multi-case families with at least two living first degree relatives with RA revealed a significantly increased prevalence of Pi Z heterozygotes (11%) compared to an expected prevalence of 1.7% (Ollier et al, 1988). A large study of 285 Newcastle RA patients (Paspiha SS, 1989) observed no relationship between the α1-PI Z allele and RA. However those studied were a heterogeneous group and sub-group analysis of more severely affected individuals only included 50 cases. None of these 50 individuals were Pi Z phenotype. This study, however, was the only study to investigate the different the different α1-PI M alleles and observed a relationship between Pi and RA. This is a surprising finding when one considers that the different Pi M alleles do not differ in their contribution to the amount of α1-PI in the serum or in their respective EIC. I have tabulated the findings of the studies listed above (table 2) and combined the data.

Table 2. Pi phenotype in RA patients and controls.

Pi Phenotype	RA patients n(%)	Controls n(%)	Odds ratio (95% CI)
MM	1422 (88.1%)	4043 (88.5%)	0.96 (95%CI 0.8-1.2), P = 0.64
MS	104 (6.44%)	366 (8.0%)	0.79 (95%CI 0.6-1.0), P = 0.04
MF	16 (1%)	30 (0.6%)	1.51 (95%CI 0.8-2.9), P = 0.18
MZ	60 (3.7%)	71 (1.5%)	2.44 (95%CI 1.7-3.5), P < 0.0001
SZ	8 (0.5%)	10 (0.2%)	2.3 (95%CI 0.8-6.2), P = 0.08
ZZ	2 (0.12%)	2 (0.04%)	2.45 (95%CI 0.3-28), P = 0.28
Z	70 (4.3%)	83 (1.7%)	2.45 (95%CI 1.8-3.4), P < 0.0001
Total	2008(100%)	4422(100%)	

A Pearson Chi square analysis shows that there is a significant difference between the two groups for overall genotype frequencies. Chi square (DF 5) = 36.1, P < 0.0001.

Clearly, possession of the Z allele is significantly more frequent in the RA patients than controls, although it still only accounts for just over 4% of the RA patients.

The control cases were normal Caucasian controls reported in the published literature (Cook PJL, 1975).

A recent whole-genome linkage analysis of RA susceptibility loci in 252 affected siblings in the UK (MacKay K, 2002) observed linkage at 14q which codes for $\alpha 1$ alleles. This and the above data would suggest that $\alpha 1$-PI deficiency could have a role in the RA disease process.

1.5.b Oxidative damage to alpha 1-proteinase inhibitor in RA

Oxidised α1-PI is another modified form of α1-PI, which is found in inflammatory exudates at levels of >5% to 10% of total α1-PI (Sepper R, 1995, Chidwick K, 1992). Chidwick demonstrated that the elastase inhibitory activity of α1-PI in fresh samples of synovial fluid from patients with RA was reduced. This was not as a result of α1-PI deficiency in rheumatoid synovial fluid, as the elastase inhibitory ability was disproportionately depressed relative to the immuno-chemically determined concentrations of α1-PI. Zang Z, 1993 reported that synovial fluid α1-PI is inactivated during exercise of the knee-joints of RA patients. Zang showed a marked decrease in the mean activity of α1-PI after exercise with no change in the molecular forms of α1-PI and suggested that oxidation may contribute to α1-PI inactivation as a consequence of 'hypoxic-reperfusion' injury after exercise of the inflamed joint. Abbink JJ, 1993 demonstrated that α1-PI in RA joints is inactivated in part by activated neutrophils, suggesting a role for these cells in impairment of the local balance between proteinases and their inhibitors in arthritis.

α1-PI contains methionine at the "P1 position," which is located in the reactive site, and determines the specificity of inhibition. Various oxidant radicals such as peroxide, the hydroxyl radical, hypochloride, chloramine, and peroxynitrite, (Wallaert B, 1991 and Vogt W, 1995) change the methionine into methionine sulfoxide, and such modified forms of α1-PI lose the inhibitory activity against NE. Furthermore, such an oxidized form of α1-PI has the function of monocyte activation (Moraga F, 2000) Thus, oxidized α1-PI promotes tissue destruction not only by the loss of proteinase inhibitory activity but also by recruiting and activating monocytes to the site of inflammation.

Further work by Chidwick, 1994 demonstrated that TNF α concentrations correlated with the extent of α1-PI inactivation and postulated that NE had a role in TNF α release within the inflamed joint.

Therefore the oxidation of α1-PI in RA may be of critical importance to the integrity of the connective tissue of the joint. Firstly uninhibited NE may directly be damaging to type II collagen, secondly oxidized α1-PI recruits monocytes to the inflamed joint, and thirdly TNF levels may be increased secondary to increased oxidized α1-PI levels.

1.5.c Immunoglobulin A-alpha 1 proteinase inhibitor serum complex in RA

The reactive centre of α1-PI is crucial to its ability to inhibit NE (Taggart C, 2000). This reactive site contains a methionine group that is rapidly oxidised and therefore inactivated by potent antioxidants such as hypochlorous acid (reviewed by Evans M, 1994). Hypochlorous acid can be produced by a neutrophil enzyme: myeloperoxidase (MPO) in combination with hydrogen peroxide (H_2O_2) via the MPO - H_2O_2 -chloride system (Weiss SJ, 1987). It has been demonstrated that oxidation of α1-PI by this system promotes binding to immunoglobulin A and the formation of IgA-α1 PI complexes (Scott LJ, 1999). These complexes form as a result of disulphide binding of α1-PI to the penultimate C-terminal cysteine residue of the IgA C-α3 domain which normally binds J-chain (Scott LJ, 1999).

The IgA-α1 PI complex has been reviewed in rheumatic diseases (Lacki JK et al, 1996). The authors of this review comment that IgA-α1 PI complexes have been observed to be raised in a number of rheumatic diseases. In 50% of SLE patients, levels of the complex are increased, particularly in those with current central nervous system involvement. Similarly, in approximately 50% sera derived from RA patients they are also found to be higher and also in patients with ankylosing spondylitis and multiple myeloma.

Raised levels of the complex correlated significantly with anatomical progression of RA (Lacki JK, 1995). The authors could not exclude the possibility that the constant high level of IgA-α1 PI may cause worsening bone erosions. A good correlation has been established between IgA and IgM RF ($r = 0.61$, $p < 0.01$), in a group of Polish RA patients (Lacki, 1994).

In an English study seropositive, erosive and nodular RA cases have significantly higher serum IgA-α1 PI complex levels than both seronegative RA cases and healthy controls (Scott LJ, 1998). Importantly RA cases with a high serum IgA-α1 PI complex level have a

greatly reduced inhibitory capacity to NE (Scott LJ, 1999). In view of the potential role of NE in the development of rheumatoid erosions and the literature concerning cigarette induced oxidation of $\alpha 1$ PI (Evans M and Prior, 1994) this raises the possibility of a mechanism by which cigarette smoking potentially worsens RA disease. Measurement of IgA-$\alpha 1$ PI serum complexes is a method of quantifying oxidative damage to $\alpha 1$ PI.

1.6 Mechanisms protecting against oxidative damage to alpha 1- proteinase

The joint fluid contains low-molecular weight antioxidants, such as ascorbate, methionine, uric acid, vitamin E and glutathione (Kurz B, 1997). Glutathione is a tripeptide (gamma-glutamyl cysteinyl glycine) in which glutamate is linked to cysteine through its gamma-carboxyl. It is synthesised in two sequential steps (Lu SC, 1999) and is present in fairly high concentrations in most animal cells (1-2 Mm; up to 8mm in liver).

Glutathione is involved in the elimination of reactive electrophiles and in conjugation to lipophilic compounds, to increase their solubility before they are excreted. These detoxyfication reactions are catalysed by glutathione S-transferases, which exist in at least ten isoenzyme forms with different substrate specificities (Strange, 2001).

Physiologically by far the most potent inhibitor of α1PI function is hypochlorous acid (HOCL) (Shock A, 1988). Activated neutrophils contain the enzyme myeloperoxidase, which uses hydrogen peroxide to oxidize chloride ions into a powerful oxidant that has been identified as HOCL (Weiss SJ, 1986). Glutathione is a ubiquitous antioxidant and has an important role in preventing oxidation of the α1-PI reactive site and this process is thought to be as a result of glutathione scavenging hydrogen peroxide (Gressier B, 1994). Conversely superoxide dismutase catalyses the conversion of the superoxide radical to hydrogen peroxide and it has been recently demonstrated important differences in the severity of erosive RA between individuals with differing manganese superoxide dismutase polymorphisms (Mattey DL, 2000).

Other systems involved in the scavenging of hydrogen peroxide include both catalase and glutathione peroxidases which break down hydrogen peroxide, reviewed in (Mates M, 2000; Arthur JR, 2000). Interestingly severe RA is associated with low glutathione peroxidase activity, reviewed in (Tarp U, 1994).

1.7 Glutathione S transferase Mu 1

The GSTs are a widely expressed group of enzymes that catalyse the detoxification of xenobiotics via glutathione conjugation, and are also believed to play an important role in detoxifying products generated by the activity of reactive oxygen species (ROS). There is evidence that some allelic variants are associated with differences in detoxification efficiency, and various cancer studies have suggested that polymorphisms in GSTM1 and GSTT1 may influence the ability to detoxify chemicals in cigarette smoke (Rebbeck TR, 1997;Seidergard J, 1990;Chenevix-Trench, 1995).

Glutathione S-transferase genes encode a family of dimeric phase 2 enzymes (monomer molecular mass 17-28 kDa) that have a pivotal role in catalysing the conjugation of glutathione to a wide range of electrophilic substrates, many of which are formed during phase 1 metabolism (Rushmore and Pickett, 1993). This is an important pathway of protection against chemical toxins. Four cytosolic GST gene families expressed in mammalian tissues have been extensively studied; alpha, mu, theta and pi. Each of these GST families may include multiple genes, with active enzymes comprising homo- and heterodimeric combinations of monomers encoded by genes of the same family. For example, 5 mu class genes are arranged in tandem on chromosome 1. Members of the same gene family show at least 65% amino acid identity (Mannervik et al., 1992). A major function of the cytosolic enzymes is believed to be the detoxification of reactive mutagenic electrophiles (Jacoby, 1978). They are also increasingly considered to have an important role, via their glutathione-dependent peroxidase activities, in free radical scavenging in vivo thus protecting cells from the deleterious effects of oxidative stress (Hayes, 1995). Therefore, these enzymes may play a role in protection against the consequences of oxidative stress. These enzymes are thought to have evolved as an adaptive response to environmental insult and utilise a wide variety of structurally diverse substrates, many of which induce GST expression (Rushmore and

Pickett, 1993). For example, GST expression is significantly increased on exposure to chemoprotective agents such as food preservatives (Smith et al., 1995). This increased expression is mediated in part by an anti-oxidant responsive element present in the promoter region of the GST genes (Rushmore et al., 1990). GSTs exhibit higher levels of constitutive expression in a variety of tissues, many of which demonstrate different patterns of GST iso-enzyme expression.

Polymorphic loci have been identified in all cytosolic GST gene families but the molecular basis and the pathological consequences of such a variation is unclear (Smith et al., 1995). Similarly, two polymorphisms at the GSTP1 locus have been identified but their significance remains unknown (Harada et al., 1994). Most interest has focused on the polymorphisms at the GSTM1 and GSTT1 gene loci.

Common null alleles have been identified at GSTM1 and GSTT1 (Ketterer et al 1993). Thus, GSTM1 demonstrates phenotypes that arise from combinations of the GSTM1*0, GSTM1*A and GSTM1*B alleles. GSTM1*0 is a complete gene deletion and homozygotes (GSTM1 null) who comprise about 50% of most populations (Benhamou S et al, 2002), express no protein.

GSTM1 has been studied particularly in relation to smoking-related cancers especially lung. Some studies have found the GSTM1 null genotype to be more common (Seidegard et al., 1990; Zhong et al., 1991) while others found no such association (Benhamou S et al, 2002). Studies in smokers with lung cancer showed that p53 and H- ras mutations were more frequent in GSTM1 null individuals suggesting GSTM1 protects these genes against mutagenesis (Ryberg et al., 1994).

1.7a Glutathione S transferases in rheumatic diseases

The role of the GST M1 polymorphism has been investigated only once in RA. The GST M1 A/B phenotype was significantly less common in RA cases than in controls (Matttey et al, 1999). This suggests that a double dose of the GST M1 gene was protective for RA. Alternatively considering those individuals who were GST M1 null the Larsen score for joint damage was higher than those RA patients with other GST M1 genotypes and this was found to be independent of the shared epitope. In fact the difference in Larsen scores between patients homozygous or negative for the shared epitope was similar to that between GST M1 null and non-null patients. There was no relationship observed between other GST genotypes and markers for disease severity. In this study a potential relationship between GST M1 null and seropositivity or the potential relationship between cigarette smoking and GST status was not considered. This may be important as it is known that GSTM1 null is associated with Ro antibody positive photosensitive Systemic Lupus Erythematosus (Ollier, 1996) suggesting that detoxification of products of oxidative stress may be important with regards to subsequent antibody formation. Morinobu A et al, 1999 demonstrated that The GSTM1 homozygous null genotype could be a genetic factor that determines susceptibility to Sjogren's syndrome and may be involved in antibody production. Tew MB et al, 2001 demonstrated that while M1 and T1 null genotypes were not significantly increased in scleroderma compared to ethnically matched controls, their frequencies were significantly higher among scleroderma patients with hypertension and pulmonary involvement. This suggests that GST genotype may be a genetic factor that contributes to clinical disease expression in scleroderma.

1.8 Aims and objectives of this thesis

Rheumatoid arthritis (RA) is a common disease with a prevalence of 1%. It is an important cause of disability. Therefore any environmental factors that can be potentially modified and play a part in RA susceptibility and or severity are of great importance. Rheumatoid arthritis is subdivided into seropositive and seronegative cases. Seropositivity is defined by the presence of RF in the sera. It has been long recognised that seropositive RA confers a poor prognosis with the development of erosive joint disease and disability compared to seronegative RA cases. Recent research has demonstrated a strong association between seropositive RA and cigarette smoking, a relationship between pack years of cigarettes smoked and RF levels in RA cases and that cigarette smoking to be an important risk factor for the development of serum RF in healthy individuals.

This thesis focuses on the relationship between cigarette smoking and RA requiring hospital outpatient follow up and the possible mechanisms by which cigarette smoking could cause rheumatoid joint damage. In the introduction, I have reviewed the natural history of RA. I have reviewed the relationship between cigarette smoking and susceptibility to the development of RA and the relationship between cigarette smoking and disease severity.

It is known that cigarette smoking is associated with RA susceptibility, however, it is not known if there is a dose relationship between cigarettes smoked and RA requiring hospital follow up. I plan to investigate this potential association by quantifying the number of cigarettes smoked by RA patients attending hospital outpatients and under taking a case control study with matched controls. Additionally I will study the relationship between cigarette smoking and a family history of RA in terms of disease susceptibility and age of onset of RA.

Potential mechanisms aside from the generation of RF underlying the relationship between cigarette smoking and RA are currently unknown. To study these potential

mechanisms I have investigated the interaction between the GST M1 genotype and cigarette

smoking, the relationship between oxidative damage to α1-PI, cigarette smoking and the

GST M1 genotype.

A specific list of hypotheses include

1) A relationship between pack years smoked and RA.

2) An increased prevalence of a family history of RA in non-smoking RA patients.

3) A genetic-environmental interaction between GST M1 null genotype and cigarette smoking in terms of RF production, rheumatoid joint damage and oxidative damage to α1-PI.

4) An association between smoking, family history of RA and the age of onset of RA.

5) An association between smoking and oxidative damage to α1-PI.

Chapter 2

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Methods

Patient selection

The patient selection in the work undertaken for chapters three, four, five and seven involved patients attending rheumatology outpatients clinics at both Whiston and St. Helen's hospitals and also the University Hospital Aintree. All patients had a consultant diagnosis of RA and met the ACR criteria for RA (Arnett, 1988).

In chapter five the RA patients were selected to represent a spectrum of clinical disease with approximately equal numbers of non-erosive and erosive cases.

In chapter six the RA patients represented a well characterised group of RA attending the Haywood Hospital Stoke-on-Trent. The patients fulfilled the ACR criteria (Arnett, 1988) and were recruited in a clinic established to examine the effects of slow acting anti-rheumatic drugs.

Criteria for family history of RA

A positive family history of RA was defined according to strict criteria. 1). A first or second degree relative with a history of RA. 2). This relative must have been under regular follow up in a rheumatological department, received a disease modifying anti-rheumatic drug, or had the deformities or nodules typical of RA. 3). As the population in this region is relatively static, case notes of the relative were checked to verify the history of RA where possible.

Cigarette smoking history

A comprehensive past and current smoking history was taken from time of inclusion to the study. Cigarette consumption was quantified in pack years (PY). One PY is equivalent to 20 cigarettes smoked per day for one year. A current smoker was taken to be a smoker of at least 1 cigarette per day for 1 year. An ex-smoker was defined as an individual who had stopped smoking for at least 3 months.

Selection of control patients

Control cases recruited in chapter three were seen in a North Staffordshire dermatology out-patients clinic. Specifically the cases all had benign non- inflammatory skin lesions. They were investigated in an identical manner to the RA patients (by Dr John Lear). Control cases included in chapters five and seven consisted of healthy hospital workers at the University Hospital Aintree.

Evaluation of social class.

The Office of National Statistics Classification of Occupations (Office of Population Censuses and Surveys, 1980)) have defined social class. The social class was based on the highest employment history of the individual prior to age of onset of disease or in the case of a female, who had never worked, by her partner's occupation. This classification allocates individuals into 1 of 6 social classes according to occupation as follows: I =professional, II=managerial, III N=non-manual skilled, or III M=manual skilled, IV=partly skilled and V=unskilled.

In chapter 6 the Carstairs deprivation index (Carstairs V, 1995) was used to categorise the socio-economic status of the patients. Carstairs scores were obtained from the 1991 census small-area statistics for the United Kingdom (UK) and assigned to each patient based on their enumeration district of residence identified from the postcode address. The index is based on a composite of four variables; male unemployment, social class of the head of the household, overcrowding and access to a car. The Carstairs scores in the RA population for this study ranged from -4.6 (least deprived) to 5.81 (most deprived).

IgA-α1PI complex levels.

A single 10ml venous sample was taken from each case. The blood was centrifuged for five minutes and the plasma stored at -70^0C. The IgA-α1 PI complex levels (arbitrary units, au) were determined using a sandwich ELISA. A reference serum from an individual with high levels of IgA-α1 PI complex was used to obtain a standard curve from which arbitrary units (a.u.) were obtained.

ELISA for IgA α1-PI

The levels of IgA-α1-PI (arbitrary units, au) were measured in serum and synovial fluid using a newly developed sandwich ELISA. Ninety-six well microtitre plates were coated overnight at 4°C with monoclonal anti-α1AT antibody (1 μg/ml; Calbiochem-Novabiochem, Nottingham) in bicarbonate buffer (pH 9.6). Plates were washed with buffer consisting of phosphate-buffered saline (PBS) and 0.05% Tween (PBS/Tween), followed by blocking of non-specific binding with 1% bovine serum albumin in PBS/Tween at 37°C for 1 h. Each patient's serum was diluted 1:50 in blocking buffer was added in duplicate (100 μl/well) and incubated for 2 h at 37°C. High and low control sera were added to each plate. After further washing in PBS/Tween, peroxidase-conjugated anti-human IgA (1:500; Sigma, Poole) was added at 100 μl/well and incubated for 2 h at 37°C. The anti-IgA antibody was reported to be specific for human IgA α chain and did not react with human IgG or IgM by ELISA or Ouchterlony double diffusion. The plates were washed in PBS/Tween and incubated with 100 μl/well of *o*-phenylenediamine dihydrochloride (OPD) in phosphate-citrate buffer (pH 5.0) plus hydrogen peroxide. After colour development for 20 min, the reaction was stopped with 50 μl of 1 molar sulphuric acid and the absorbence read at 492 nm

in a Titertek Multiskan Plus MK II plate reader (Labsystems). A reference serum from a RA patient with high levels of IgA-α1-PI complex was used to obtain a standard curve from which arbitrary units were determined. A 1:50 dilution of the reference serum was used as the highest standard (100 au) and further dilutions of this were used to produce the standard curve over the range 2.5-100 au. The curve was approximately linear over the range chosen and illustrates a significant difference in OD between the highest and lowest standards. Sera diluted at 1:50 was taken since this was the optimum dilution for most sera to give values on the reference curve. The intra- and inter-assay coefficients of variation were 9.6 and 13.9%, respectively.

Statistical analysis

Conditional logistic regression was used to calculate the odds ratio (OR) for having RA. Between RA patients and matched controls, frequencies were compared using the Sign test for categorical data and the Wilcoxon-Signed Rank test for continuous data. Between groups within RA patients, frequencies were compared with the chi-squared test and continuous data using the two-sample t-test where a Gaussian distribution could be assumed or the Wilcoxon two-sample test otherwise (indicated by the quoting of medians rather than means). Statistical significance was set at the 5% level.

The association of disease severity measures with GST genotypes and smoking was assessed using multiple regression analyses with adjustment for the independent variables, age, and disease duration where appropriate. The influence of rheumatoid factor (RF) and the Carstairs deprivation index were also examined by inclusion as independent variables in some of the regression models. Additional analyses were carried out on the patient population stratified by GST status. In some analyses we also examined the effect of interaction between variables using multiple regression models that contained the interaction term as well as the corresponding main effects. Where appropriate correction for potential

multiple testing errors was performed using Holm's procedure (Holm S, 1979). All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v.6.0.4), or the PEPI software package (v 2.0) for epidemiological analysis (Gahlinger PM, 1995).

The relationships between IgA-α1PI complex levels and smoking status, erosive RA and RA disease activity were examined by multiple regression analyses with correction for the independent variables, age, gender, and disease duration where appropriate. In cases where variables were not normally distributed they were transformed to normality by using the log values. Multivariate logistic regression analysis was used to determine the variables most closely associated with erosive RA. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v.6.0.4).

Phenol/Chloroform Extraction of Genomic DNA from Blood

Part 1

- Cold lysis buffer was added to 3ml EDTA blood in a 50ml centrifuge to give a final volume of 35ml, mixed by inversion and centrifuged at 2000 rpm for 10 mins.

- The supernatant was decanted to waste.

- The white nuclear pellet was re-suspended in 1.5ml cold SE buffer and 170 ml of a

- Solution containing SDS (5% w/v) and proteinase K and incubated for 18h at 37°C.

Part 2

- 1.5ml phenol (tris-HCR saturated, pH 8.0, containing 8-hydroxyquinoline) was pipetted into glass tubes.

- 1.5ml chloroform:isoamyl was pipetted into two further sets of the labeled glass tubes.

- The proteinase K-treated samples were transferred into the phenol-containing tubes, mixed, placed in Falcon containers and spun at 2000 rpm room temperature for 15 mins in sealed buckets.

- The viscous aqueous layer was taken off into a chloroform-containing glass tube, mixed well and spun at 2000 rpm, room temperature for 5 mins. The viscous aqueous layer was taken and the chloroform:isoamyl alcohol extraction repeated. The aqueous layer was transferred to a fourth set of tubes and 0.1 volume (150μl) Sodium Acetate (see appendix) and 2 volumes (3.3ml) Ethanol were added.

- The tube was inverted tube until the DNA precipitates and DNA removed with a glass hook. The DNA was re-suspended in 200μl of sterile water and dissolved at 4°C.

GSTM1 Genotype Determination. PCR of GSTM1 (using the amplified refractory mutation system (ARMS) method

Determination of the GSTM1 genotype was performed by PCR on ARMS, using the following oligonucleotide primers:

Common GSTM1 specific

| Exon 4 forward | 5'-CTGCCCTACTTGATTGATGGG-3' |
| Exon 5 reverse | 5'-CTGGATTGTAGCAGATCATGC-3' |

GSTM1 allele specific

Intron 6 forward	5'-GCTTCACGTGTTATGAAGGTTC-3'
Exon 7 (A-specific)	5'-TTGGGAAGGCGTCCAAGCGC-3'
Exon 7 (B-specific)	5'-TTGGGAAGGCGTCCAAGCAG-3'

B-globin (internal control)

| B-globin (forward) | 5'-ACACAACTGTGTTCACTAGC-3' |
| B-globin (reverse) | 5'-CAACTTCATCCACGTTCACC-3' |

1.) DNA samples, including known *GSTM1*A* and *GSTM1*B* positive controls, Taq polymerase and 10x buffer (10mM Tris-HCl, pH 9.0, 0.1% v/v Triton X-100, 1.5mM MgCl), dNTP's and primers were removed from the -20°C and allowed to reach room temperature.

2.) Two 0.5 ml microcentrifuge tubes were labeled "A" and "B" respectively for each test DNA sample. One "A Stock" 1.5 ml tube and another "B Stock" were marked. Two tubes for positive controls for *GSTM1*A* and *GSTM1*B* were labeled, as well as, two other tubes for negative controls.The "A" and "B" Stock tubes contained the following:

	"A Stock"	"B Stock"
Sterile water	216ml	216ml
10x Taq buffer	100ml	100ml
Exon 4/5 primers	200ml	200ml
Intron 6 primer	100ml	100ml
A-specific primer	100ml	------
B-specific primer	------	100ml
b-Globin primer (forward)	100ml	100ml
b-Globin primer (reverse)	100ml	100ml
d-NTP's	40ml	40ml
Taq polymerase	4ml	4ml

3.) All these components of the "A" and "B" Stock solutions were mixed gently by inversion and 48ml of each was aliquoted respectively to every "A" and "B" labeled tube. To both negative control tubes 1.5ml of sterile water was added, whereas *GSTM1*A* or *GSTM1*B* positive DNA was added to the tubes labeled as *GSTM1*A* and *GSTM1*B* positive control. Each "A" and "B" marked tubes received 1.5ml of test DNA. To every tube two drops of mineral oil were added. Finally these tubes were centrifuged at 12000rpm for 20 sec.

4.) The tubes were randomly placed in a thermal cycler (Omnigene Hybaid Ltd.) and incubated for one cycle of 150 sec at 94°C, followed by five consecutive cycles at 94°C for 45 sec, 60°C for 60 sec and 72°C for 60 sec. Subsequently thirty cycles at 94°C for 30 sec, 58°C for 30 sec and 72°C for 45 sec. The final cycle consisted of 5 sec at 40°C.

5.) Samples were resolved by electrophoresis (120V. 45 min.) in 4% agarose gels, containing Ethidium Bromide (0.5mg/l) and photographed under U.V. light on a transilluminator.

Chapter 3

Heavy cigarette smoking is strongly associated with RA particularly in

those without a family history of RA

Introduction

Whilst both genetic and environmental factors are thought to contribute to the development of RA (Lawrence JS, 1970), the aetiology of this disease remains unclear. The genetics of RA are complex (Ollier WER, 1995). At the simplest level, there is an increased risk of hospital based RA if a family member also has the disease, odds ratio (OR) = 6. (Koumantaki Y et al, 1997). The natural history of RA, however, suggests that the disease may be initiated by relatively contemporary environmental factors. Rheumatoid arthritis was first described as recently as 1800 and examination of the visual arts before this time has failed to show any evidence of RA (Fischer KM, 1991). The low concordance for RA in monozygotic twins (Seldin MF, 1999) highlights the importance played by environmental factors.

One potential environmental agent is cigarette smoking. A Norwegian study reported that men who were current smokers had an increased risk of developing sero-positive RA (OR 4.77 95% CI 2.09 to10.9) (Uhlig T, 1999). However, this study, did not quantify the number of cigarettes smoked and therefore could not address the potential effects of cumulative exposure to cigarette smoke. In other smoking related diseases, such as emphysema (Khoury MJ, 1986), lung cancer (Doll R, 1994) and ischaemic heart disease (Howard G, 1998) there is an increased risk of disease with increasing cumulative exposure to cigarette smoke. In a North American study of female health workers, an increased risk of 49% for developing sero-positive RA was found in those smoking 25 cigarettes per day for more than 20 years (Karlson EW, 1999). Both the above studies investigated community - based RA where there is a lower prevalence of disability than in hospital- based RA cases (Jonsson B, 1990). This is relevant as current cigarette smokers of 25 PY or more are 2.4

times more likely to have erosive RA rather than the milder non- erosive RA than individuals who have never smoked (Saag KG, 1997).

I therefore undertook a study to test the hypothesis that a high cumulative exposure to cigarettes is associated with RA requiring hospital out-patient treatment, and to examine the potential role of a positive family history of RA underlying this.

Methods

Two hundred and thirty nine unrelated patients with RA (age range 28-87) attending rheumatology clinics in two Merseyside hospitals and fulfilling the 1987 ARA criteria for RA (Arnett FC, 1988) were studied. The patients' age and age of disease onset were recorded. Social class was defined by the Office of National Statistics Classification of Occupations (HMSO, 1980) based on the highest employment history of the individual prior to age of onset of disease or in the case of a female who had never worked, by her partner's occupation. This classification allocates individuals into 1 of 6 social classes according to occupation as follows: I =professional, II=managerial, III N=non-manual skilled, or III M=manual skilled, IV=partly skilled and V=unskilled. A positive family history of RA was defined according to strict criteria. 1). A first or second degree relative with a history of RA. 2). This relative must have been under regular follow up in a rheumatological department, received a disease modifying anti-rheumatic drug, or had the deformities or nodules typical of RA. 3). As the population in this region is relatively static, case notes of the relative were checked to verify the history of RA where possible.

A comprehensive past and current smoking history was taken from time of inclusion to the study. Cigarette consumption was quantified in pack years (PY). One PY is equivalent to 20 cigarettes smoked per day for one year. A current smoker was taken to be a smoker of at least 1 cigarette per day for 1 year. An ex-smoker was defined as an individual who had

stopped smoking for at least 3 months. Control cases were seen in a North Staffordshire dermatology out-patients clinic. Specifically the cases all had benign non- inflammatory skin lesions. They were investigated in an identical manner to the RA patients (by JL). The RA patients and controls were then matched for age, sex and social class.

Statistical analysis

Conditional logistic regression was used to calculate the odds ratio (OR) for having RA. Between RA patients and matched controls, frequencies were compared using the Sign test for categorical data and the Wilcoxon-Signed Rank test for continuous data. Between groups within RA patients, frequencies were compared with the chi-squared test and continuous data using the two-sample t-test where a Gaussian distribution could be assumed or the Wilcoxon two-sample test otherwise (indicated by the quoting of medians rather than means). Statistical significance was set at the 5% level.

Results

There were 239 RA cases, with a mean (SD) age was 60.5 years (11.78). The demographic details are summarised in Table 3.

Case control study: Smoking status at time of study.

RA patients were significantly more likely to be current smokers than controls, matched for age, sex and social class (table 4). Of the RA patients, 100 (42%) were current smokers vs 52 (22%) of the control cases (p<0.0001). There was no significant difference in the proportion of cases being ex-smokers, 55 (23%) of the RA cases vs 72 (30%) of the controls (p=0.09). Of the RA cases, significantly fewer had never smoked 84 (35%) vs 115 (48%) of the control s, (p=0.005).

There was a highly significant difference between pack years smoked in the RA cases and controls (a median value of 18.0 vs. 1.0, p<0.001.).

There was a dose dependant association between PY smoked and RA (summarised in table 5). The association between RA and ever having smoked and RA was modest (matched OR 1.81, 95% C.I. 1.22 to 2.19, p=0.002), whilst there was a strong association between heavy cigarette smoking and RA with 40-50 pack years (matched O.R. 13.54 95% C.I. 2.89 to 63.38, p<0.001).

Comparison of smoking between RA cases with or without a family history of RA.

There were 143 (60%) RA cases with no family history of RA (FH-ve RA). Comparing these patients with the RA cases with a positive family history of RA (FH+ve RA) there were no apparent differences in age, sex or social class. These details are summarised in Table 6. A comparison of the smoking history was made between these 2 groups. The RA FH -ve cases smoked significantly more than RA FH+ve cases. More RA FH-ve cases had ever smoked (72% vs 54%, p=0.006), were smoking at the time of disease onset (58% vs 39%, p=0.003) and had smoked greater pack years (median of 25.0 vs 4.0, p<0.001). The median RA FH-ve cases cumulative exposure to cigarettes was a 25.0 PY more than the dermatology disease controls (25.0 RA vs 0.0 controls; p<0.001), whilst there was no difference for RA FH+ve cases (4.0 PY vs 5.0 controls; p=0.980). Furthermore, the mean age at onset of RA FH+ve cases was significantly lower (44 yrs; (12.5), median age 43, range (18-73) than the FH-ve group (mean age 49 yrs; (13.5), median age 50, (19-78), p=0.007). The disease duration was longer in RA FH+ve cases (mean 17.6 years (9.5)) as opposed to RA FH-ve cases (mean 10.8 (9.9) years, p<0.0001).

Comparison of RA cases never having smoked and RA cases smoking at the time of disease onset.

There were 118 (49%) of the RA cases currently smoking at the time of disease onset. Eighty five (35%) of the RA cases had never smoked. The mean age of onset differed significantly between these two groups, 43.2 years (13.8) in the RA cases never having smoked (median age 42 years, range 18-70) in comparison to a mean age of 47.6 (12.3) (median age 47 years, (18-72)) in the RA cases smoking at the time of disease onset, (p=0.001). Significantly more of the RA cases, 46 (54%) who had never smoked, had a positive family history of RA, as opposed to 39 (33%) of the RA cases smoking at disease onset, (p=0.004).

Discussion

In this study, the relationship between cigarette smoking in RA cases attending rheumatology outpatient clinics in Merseyside, an industrial region of the North of England has been examined. A dose dependant association between PY smoked and RA was observed. A high cumulative exposure to cigarette smoke was associated strongly with RA. Secondly the RA patients with a family history of RA were significantly less likely to be current cigarette smokers at the time of disease onset and smoked significantly less PY than the RA group without a family history of RA.

The inclusion of control cases from a different region of the North of England is unlikely to have introduced a bias as the control cases were matched for age, sex and social class which are the principal determinants of the prevalence of cigarette smoking (Office of National Statistics, 1998).

Secondly it is possible that dermatology patients smoke in a different fashion than the general population. I feel this is unlikely as we excluded patients with inflammatory skin lesions such as psoriasis, a condition associated with excess tobacco consumption (Mills CM, 1992) and atopic eczema which is associated with asthma and therefore disassociated

from heavy cigarette smoking. Control cases with malignant skin lesions were also excluded, as these are observed significantly more frequently in outside manual workers, such as agricultural workers (Rosso S, 1999) and welders (Tenkate TD, 1999). These particular occupations may not be representative in terms of their smoking history in comparison to manual indoor workers who may have smoking constraints imposed upon them. Furthermore, the control cases matched the RA +ve FH group in terms of PY smoked. This is important as the smoking history of the RA +ve FH group is typical of that of the adult population aged 18-60 years for our region of the North West of England. Thirty nine per cent of the RA +VE FH group smoking at the time of diagnosis, compared to thirty-nine per cent of adults (18-60) who are current smokers in our region of Merseyside (St. Helens) (Office of National Statistics, 1998).

I have confirmed previous findings that cigarette smoking per se is modestly associated with RA. For ever having smoked, the association with RA is modest, matched OR (1.81, 95% CI 1.22,2.19). This is a similar association to that observed by (Symmons, 1997) in a *community- based* study (OR 1.66, 95% CI 0.95-3.06). However the number of cigarettes smoked was not quantified in Symmons's study, or in other studies (Uhlig T, 1999 and Heliovaara, 1993) investigating the association between cigarette smoking and RA. Therefore no direct comparison can be made between these studies and my own observations regarding the risk for heavy cigarette smoking. My study demonstrates the need to quantify the number of cigarettes smoked when studying the relationship between cigarette smoking and its association with RA. The strong association with a high cumulative exposure to cigarettes may partly underlie the observation that the incidence of RA increases with age. In keeping with this hypothesis, a Finnish study observed a twenty fold increased incidence of RA in men currently smoking as opposed to men who had never smoked after individual follow up of 14 or more years (Heliovaara, 1993).

I have observed a modest non- significant association between smoking and RA, in individuals who had smoked less than 30 PY. However, I observed that prolonged exposure to cigarette smoke resulted in a strikingly increased risk of developing RA for patients with a 40-50 pack year cigarette habit. This has implications for studies in countries where heavy cigarette smoking is extremely uncommon. An example of this is a large prospective study over 20 years in Finland designed to examine the relationship between cigarette smoking and the development of RA (Heliovaara, 1993). The study observed no relationship between cigarette smoking and RA in Finnish women. However, only 2.5% of the women studied were current cigarette smokers of more than 15 cigarettes per day and few would be exposed to more than 30 PY of cigarettes.

In contrast, a recent large North American *community based* study did observe a significant increased risk of RA in women smoking more than 25 cigarettes per day for more than 20 years (Karlson EW, 1999). The increased risk observed in the North American study is modest, but this study had a low response rate (22%) and were self diagnosed. Non-responders in RA studies tend to be older (Uhlig T, 1999), have greater co-morbidity and are poorer than responders (Rupp I, 2002). It is likely that smokers are over represented in those particular non-responder groups and therefore the association with RA might have been underestimated.

It is noteworthy that my findings apply to the more severe RA cases seen in patients attending out-patients clinics, and are not necessarily true for milder, community-based RA. In this study group the prevalence of seropositivity was approximately 90%. The majority of studies investigating the association between cigarette smoking and RA have been community based studies (Uhlig T, 1999; Karlson EW, 1999; Symmons DP, 1997; Heliovaara M, 1993; Voight LF, 1994).

Genetic factors are of clear importance in the development of RA. Family history of RA was chosen as a simple surrogate marker for genetic predisposition. The criteria for "RA" in relatives were made as stringent as possible, to avoid the common mistake of calling a degenerative disease "rheumatism" or "RA". Forty per cent of our RA population had a family history of RA. This is higher than previously reported in the literature, however these studies included first degree relatives only (Seldin MF, 1999).

I observed that RA FH+ve cases had an almost identical PY history to their respective controls and smoked significantly less than the RA FH-ve cases. On average the RA FH+ve had RA longer (6.8 years) than RA FH-ve cases. In keeping with this finding, RA cases with disease-associated MHC genes appear to develop RA before those who do not (Gran JT, 1983). These data would also suggest that RA does not predispose patients to smoke more heavily as has been previously suggested (Deighton C, 1997) as the FH+ve group had RA the longest but smoked the least. My observation that RA cases that have never smoked have a significantly increased prevalence of a family history of RA compared to those that smoke at the time of diagnosis would affect the interpretation of the many studies that have investigated the genetics of RA without recording the smoking history.

The reasons why prolonged cigarette smoking should be strongly associated with the development of RA are not clear. A recent study by Wolfe, 2000 observed that RF concentration in a group of RA cases was strongly related to the number of PY smoked irrespective of the smoking status of the patient at the time of the study. A longitudinal community study of healthy individuals observed that the persistence of RF significantly increases the risk, 7.5 fold for the development of RA (Halldorsdottir, 2000) and there is certainly a strong association between cigarette smoking and RF production in healthy individuals (Tuomi T, 1990). I hypothesise that prolonged heavy cigarette smoking, but not smoking per se results in increased RF production, and that in part this explains the

relationship of increasing pack years smoked and the association with RA. Surprisingly there have been no studies of other possible mechanisms to explain the association between cigarette smoking and RA.

This study may in part explain the increased mortality observed in RA (Kvalvik AG, 2000), as current data show that continued cigarette smoking throughout adult life doubles age specific mortality rates, nearly trebling them in late middle age (Doll R, 1998).

Since this study was published there have been four publications on this subject. A Swedish study (Olsson et al, 2001) investigating co-morbidity and lifestyle in hospital outpatients based RA observed current and previous smoking were associated with increased risks for seropositive RA in both sexes. In both sexes there was a dose-response relationship found with number of tobacco pack years (p for trend <0.005 men and p=0.029 women.). The number of pack years smoked were banded <5, 5-9, 10-19, >20. Therefore high intensity smoking and modest smoking (>20 pack years were grouped together). Current and previous smoking were associated with increased risks for RA in both sexes, and in men a dose-response relationship was found with number of tobacco pack years (p for trend <0.005). For men smoking > 20 pack years the risk for seropositive RA was increased significantly OR 3.4 (1.5-8.4) and for women OR 2.5 (0.9-6.7).

Criswell LA, 2002 studied whether cigarette smoking increases the risk of RA among postmenopausal women. A cohort of 31 336 women in Iowa who were aged 55 to 69 years in 1986 and who had no history of RA. Through 1997, 158 cases of RA were identified. Multivariable Cox proportional hazards regression was used to derive rate ratios (RRs) and 95% CIs for the association between cigarette smoking and RA. Compared with women who had never smoked, women who were current smokers (RR = 2.0; 95% CI: 1.3 to 2.9) were at increased risk of RA. Those who had quit 10 years or less before study baseline (RR = 1.8; 95% CI: 1.1 to 3.1) were at increased risk of RA, but women who had quit more than 10

years before baseline were not at increased risk (RR = 0.9; 95% CI: 0.5 to 2.6). Both the duration and intensity of smoking were associated with RA. Multivariable adjustments for age, marital status, occupation, body mass index, age at menopause, oral contraceptive use, hormone replacement therapy, alcohol use, and coffee consumption did not alter these results.

Additionally Masi, 2001 published his investigations regarding the relationship between RA and heavy cigarette smoking. A case-control study nested within a community based cohort (n=21061 adults) enrolled in 1974. Twenty years later 54 individuals were noted to develop RA. These were matched with four controls from the entry cohort and were matched for age, sex, and race (all white subjects). Heavy cigarette smoking (>30 cigarettes per day) was associated with RA (OR 21.5, 95% CI 1.9 to 122.5, p=0.005).

A very recent Finnish study (Krishnan E, 2003) studied 1095 patients with RA and 1530 control individuals Preliminary analyses revealed the presence of substantial statistical interaction between smoking and sex (P < 0.001). In separate multivariable analyses, past history of smoking was associated with increased risk for RA overall in men (odds ratio 2.0, 95% confidence interval 1.2-3.2) but not in women. Among men, this effect was seen only for rheumatoid factor-positive RA. Interestingly in women, but not in men the smoking RA women had a significantly later age of onset than non-smoking RA women. I would contend that this because of a pack year susceptibility rather than a protective effect of smoking in female RA cases as suggested by the authors Krishnan E et al, 2003.

This study was not designed to address whether an association between cigarette smoking and RA is causal. As possible evidence, we would cite the reduced incidence of RA in the UK over recent years (Silman AJ, 1986), coinciding with an overall reduction in smoking evident over the last thirty years. In 1971 45% of the adult population were current smokers (Office of National Statistics, 1972), as opposed to 28% in 1996 (Office of National Statistics, 1998). An unfortunate corollary is that this trend may be reversed in the years to

come as the proportion of young girls smoking is increasing (Office of National Statistics, 1998). If the recent trend for increased cigarette consumption in young woman continues, a new epidemic of RA may occur, not immediately, but in the time it takes to smoke the equivalent of 30 or more PY of cigarettes.

Table 3. Characteristics of RA cases and controls studied.

	Cases	Controls
Number	239	239
Gender		
Male	23%	23%
Female	67%	67%
Age (years)		
Mean (S.D.)	60.5 (11.8)	60.5 (11.8)
Age at Onset (years)		
Mean (S.D.)	46.9 (13.2)	N.A.
Disease Duration (years)		
Mean (S.D.)	13.6 (9.9)	N.A.
Social Class		
Classes I-II	12%	12%
Classes IIIN-IIIM	36%	36%
Classes IV-V	52%	52%

Table 4. Comparison of smoking history between RA cases and dermatology controls

| | Current smokers | Ex-smokers | Never smoked | Pack years |
| | No. (%) | No. (%) | No. (%) | Median |
				(Semi-IQR) [1]
Cases *(n=239)*	100 (42%)	55 (23%)	84 (35%)	18.0 (18.5)
Controls *(n=239)*	52 (22%)	72 (30%)	115 (48%)	1.0 (8.5)
Significance	$p<0.001^2$	$P=0.09^2$	$p=0.005^2$	$p<0.001^3$

1: Semi-Interquartile Range

2: Resulting from a Sign test.

3: Resulting from a Wilcoxen-Signed Rank test.

Table 5. Odds ratio for having rheumatoid arthritis with pack years of cigarettes smoked.

Pack Years	Cases (%)	Controls (%)	Odds Ratio[1]	95% C.I.[2]	Significance
1-10	112 (47%)	161 (67%)	0.80	(0.4,1.5)	p=0.496
11-20	15 (6%)	35 (15%)	0.55	(0.3,1.2)	p=0.111
21-30	34 (14%)	26 (11%)	1.76	(0.9,3.3)	p=0.068
31-40	30 (13%)	7 (3%)	5.72	(2.3,14)	p<0.001
41-50	27 (11%)	4 (2%)	13.54	(2.9,63)	p<0.001
>50	21 (9%)	6 (3%)	8.41	(2.5,29)	p<0.001
Ever smoked	155 (65%)	124 (52%)	1.81	(1.2,2.3)	p=0.002

1: Calculated using conditional logistic regression

2: 95% confidence interval

Table 6. Comparison of demographic and smoking history between RA cases with or without a family history of RA.

	Family History +ve (n=96)	Family History −ve (n=143)	Significance
Gender			
Male	23 (24%)	29 (20%)	
Female	73 (76%)	114 (80%)	p=0.499[1]
Age at Onset (years)			
Mean (S.D.)	44 (12.5)	49 (13.5)	p=0.003[2]
Smoking at Diagnosis			
Yes	37 (39%)	83 (58%)	
No	59 (61%)	60 (42%)	p=0.003[1]
Social Class			
Classes I-II	11 (11%)	19 (13%)	
Classes IIIN-IIIM	37 (39%)	47 (33%)	
Classes IV-V	48 (50%)	77 (54%)	p=0.656[1]
Number of Pack Years			
Median (Semi-IQR.) [4]	4.0 (13.5)	25.0 (21.5)	P<0.001[3]

1: Resulting from a Chi-squared test

2: Resulting from a two-sample t-test

3: Resulting from a two-sample Wilcoxon test

4: Semi-Interquartile Range

Chapter 4

The relationship between current cigarette smoking and the age of onset of

RA in individuals with sporadic and familial RA.

Introduction

It is my impression clinically that smoking influences the age of onset of RA and that it is an important environmental risk factor for the age of development of RA. The data presented in chapter 3 of this thesis suggests a possible increased risk for developing RA for smoking >30 PY. This risk, therefore, for the average smoker smoking 20 cigarettes per day would be apparent only after thirty years. The majority of smokers start smoking in their late teenage years (Office of National Statistics, 1998). The average persistent smoker will therefore have a potentially increased risk of developing RA in their early fifties.

In contrast the data from chapter 3 suggests that RA patients who have never smoked are significantly more likely to have a family history of RA than RA smokers. I found that significantly more of the RA cases who had never smoked, had a positive family history of RA (54%), as opposed to (33%) of the RA cases smoking at disease onset, (p=0.004).

These never smoked RA patients are presumably more dependent on genetic factors as a trigger factor for their disease and are not dependent on a high pack year smoking history for disease development. I have demonstrated in chapter 3 that never smoked RA cases present at a significantly earlier age than RA patients smoking at the time of diagnosis. In the RA cases never having smoked the median age of disease onset was 42 years, range 18-70. In comparison to median age of disease onset of 47 years, (18-72)) in the RA cases smoking at the time of disease onset, (p=0.001).

However, on reviewing the literature the study of the age of onset between familial and sporadic RA cases has been made without considering the smoking history. Interestingly there have been conflicting results regarding the age of onset of sporadic and familial RA. I feel it is possible that these discrepancies may be the result of studying patient populations with a different prevalence of cigarette smoking. An example of this is a well conducted

prospective study regarding familial and sporadic RA (Radstake, 2000). In this study the age of onset of sporadic and familial RA was similar in women, but significantly different in men. In the male familial RA cases the age of onset was significantly earlier than in those without a family history of RA. No explanation is given for the differences observed between men and women regarding age of onset. However I hypothesise that a difference in prevalence in smoking between the male and female RA cases in the Dutch study is a possible explanation for the different age of onset of RA between males and females. Cigarette smoking is a particularly important environmental risk factor for RA in men, as men who are current smokers have an increased risk of developing sero-positive RA (Odds Ratio 4.77; Confidence intervals 2.09,10.9) (Uhlig T, 1999). The prevalence of ever having smoked in male seropositive RA cases is 90% (Heliovaara M, 1993) whereas studies of female RA in the Netherlands observed a much lower prevalence of smoking at approximately 40% (Hazes JMW, 1990).

Accordingly I hypothesise that the age of onset of familial and sporadic RA is different in smokers, but not in lifelong non-smokers.

To determine if cigarette smoking at the time of onset of RA influences the age of onset of familial and sporadic RA I studied RA patients smoking at the time of disease onset and RA patients who had never smoked.

Methods

Three hundred and sixty unrelated RA patients (age range 28-87), satisfying the revised ARC criteria for RA (Arnett FC, 1988) and attending rheumatology clinics in two Merseyside hospitals were studied. The patient's age of disease onset was determined from the case note records. A positive family history of RA was defined according to strict criteria. 1). A first or second degree relative with a history of RA. 2). This relative must have been

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under regular follow up in a rheumatology department, received a disease modifying anti-rheumatic drug, or had the deformities or nodules typical of RA. 3). As the population in this region is relatively static, case notes of the relative were checked to verify the history of RA where possible.

A smoking history was obtained and the patients were included in the study if they had never smoked or were smoking at the time of RA onset.

Statistical Analysis

The Mann-Whitney U-test was used to compare the ages at disease onset. A P value less than 0.05 was considered statistically significant. All data are shown as medians with the range.

Results

Age of onset of RA in familial and sporadic RA smokers

Considering those patients smoking at the time of disease onset with familial (n=90) and sporadic RA (n=134), there was a striking difference in the age at onset of RA. The median age of onset in familial RA patients was significantly earlier than the sporadic RA patients, 40 years (range) (18-72) vs. 50 years (18-72), p<0.00001.

This was true of both male and females. The median age of onset in smoking female familial RA patients (n=60) was significantly earlier than the smoking female sporadic RA patients (n=88), 41 years (18-72) vs. 50 years (18-72), p=0.002. Equally in the smoking male familial RA patients (n=30) as opposed to smoking male sporadic patients (n=46) the age of onset was again strikingly earlier 38 years (18-72) vs. 50 years (21-75), p=0.0001.

Age of onset of RA in familial and sporadic RA non-smokers

Comparing patients who had never smoked, the median age of onset of disease was similar in both groups, familial RA (n=70) median age of presentation 44 years (18-75) and sporadic RA (n=66) 46 years (19-74), p=0.6.

Age of onset of RA in sporadic RA smokers and non-smokers

Considering the smoking sporadic RA cases (n=134) there is a trend, p=0.06 for these patients to present later than the never smoked sporadic RA cases (n=66), 50 years (20-75) vs. 46 years (19-74).

Age of onset of RA in familial RA smokers and non-smokers

I observed the opposite trend to that observed in the sporadic RA cases. In the familial RA cases the median age of onset of RA in the smoking RA cases was earlier 40 years (18-72) than the never smoked cases 44 years (18-75), p=0.16, although this did not reach statistical significance. All of these data are summarised in table 7.

Discussion

I have studied a large cohort of RA patients and demonstrated a striking difference in age of onset of RA between sporadic and familial cases, but only in those who smoke at the time of disease onset. I found no difference in the age of onset between sporadic and familial RA cases that had never smoked. It is possible that, for the smoking sporadic RA cases, cigarette smoking is the principal risk factor for the development of their disease. This risk is likely to be evident following chronic exposure to cigarette smoke - as is the case with other smoking related diseases such as lung cancer (Doll R, 1994) and pulmonary emphysema (Khoury MJ, 1986) – and as such might only become evident after many years of smoking. On the other hand both smoking and a family history of RA represents a combination of risk

factors for RA and it is interesting that the group with both of these risk factors have a disease onset which is earlier than any of the other groups.

In this study I considered a family history as positive if a first or second degree relative had RA. This is in contrast to other studies investigating the relationship between age of onset of RA and a family history of disease. These studies considered a family history of RA was present if a first-degree relative had RA. I feel that my approach is valid. Recently an Icelandic study observed an increased risk of RA if a first or a second-degree relative had a history of RA (Grant SF, 2001). An individual with a sibling with RA had a risk ratio of 4.38 (95% confidence interval 3.26-5.67) for developing RA. However, an individual with an uncle or aunt with RA had a risk ratio of 1.95 (95% confidence interval 1.52-2.43) for developing RA. This study clearly demonstrated a familial component of RA extending beyond first-degree relatives.

It is possible that there is an interaction between genetic and environmental risk factors that increase the risk of developing RA at an earlier age. Radstake, 2000 observed that HLA-DR alleles coding for the shared epitope were similar, and not significantly different between patients with familial or sporadic RA. However, other putative RA susceptibility genes such as the $\alpha 1$-PI Z allele (Cox DW, 1985) are observed with greater frequency in familial RA (Ollier, 1988) compared to randomly selected RA (Abboud RT, 1991). Cox, 1985 observed that RA individuals with the Z allele develop RA at a younger age than non-Z allele RA cases. The contribution of the $\alpha 1$-PI Z allele to diseases such as pulmonary emphysema is increased significantly in cigarette smokers (Norman MR, 1997) who tend to present earlier and a similar phenomenon might occur in RA.

HLA DRB 0401 is observed in 12% of the UK population (Balsa A, 2000) and in 54% of a Stoke hospital based RA population (Mattey, 2002). This is a very important genetic factor in the development of hospital based RA (Balsa A, 2000). A recent study has observed a

significant interaction between cigarette smoking and HLA DRB1 0401 and RF production in RA patients (Mattey DL, 2002). In those RA patients smoking DRB1* 0401patients 73% were seropositive compared with 55% seropositive who were non-smokers and DRB1*0401. Of those who smoked and were not DRB1* 0401an intermediate prortion (63%) were seropositive. This highlights another potential interaction between cigarette smoking and genetic factors in RA. If an interaction exists between smoking, DRB1*0401 and RF in healthy individuals as demonstrated in RA individuals, this increased RF production may play a part in the development of seropositive RA.

Furthermore, the cytokine interleukin 1 has been observed to be important in the development of joint inflammation and the development of erosions (Feldmann M, 2001). There is evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis (Cox A, 1999). Interleukin 1 production may be stimulated by the direct effects of tobacco glycoproteins (Dinarello, 1996). The concept of competition between IL-1 and sIL-1ra for occupation of IL-1 receptors has recently generated the hypothesis that an imbalance between IL-1 and IL-1-ra may play a role in the pathogenesis of RA (Firestein GS, 1994). Alveolar macrophages from smokers with interstitial lung disease have been shown to produce lower levels of IL-1ra than those obtained from nonsmokers (Janson RW, 1993). In Graves ophthalmopathy sIL-1ra levels were negatively correlated with the number of cigarettes smoked (Hofbauer LC, 1997). Therefore genes producing high level of IL-1 and low levels of sIL-1ra may interact with cigarette smoking to create a great imbalance between IL-1 and IL-1-RA and the development of severe RA requiring hospital treatment.

I have demonstrated for the first time that the age of onset of RA differs between sporadic and familial RA cases if they smoke up until the time of disease onset where as lifelong non-smoking sporadic and familial RA cases do not differ in their age of onset.

Table 7. The influence of current smoking on the age of onset of familial and sporadic RA

RA patient type and smoking history at RA onset	Number	Median age at onset (range)	P value
Sporadic smoker	134	50 (20-75)	<0.0000
Familial smoker	90	40 (18-72)	
Sporadic never smoked	66	46 (19-74)	0.6
Familial never smoked	70	44 (18-75)	

Chapter 5

Serum complexes of IgA-α1PI in RA: Association with current cigarette smoking and disease activity

Introduction.

Rheumatoid arthritis (RA) is a heterogeneous disease, with a disease spectrum ranging from a persistent inflammatory, but non-erosive joint disease to a rapidly progressive erosive joint disease (Utsinger PD, 1985). Recently it has been suggested that cigarette smoking is an important trigger factor for the development of erosions in RA (Saag KG, 1997; Wolfe F, 2000; Mastottir B, 2000). The way in which smoking influences RA severity is unclear at present.

Rheumatoid erosions are associated with increased proteinase activity within the joint (Goldring SR, 2000). Matrix metalloproteinases (MMPs) are a class of structurally related zinc-dependent proteinases that are collectively responsible for the metabolism of extracellular matrix proteins (Birkedal-Hansen H, 1993) and are thought to play an important part in erosive RA (Brinckerhoff CE, 1991). Tissue inhibitors of MMPs (TIMPs) precisely control MMP activity (McCachren SS, 1991). TIMP 1, an important inhibitor of the specific MMPs that cause rheumatoid joint damage (Kolkenbrock H, 1991), is destroyed by neutrophil elastase (NE) (Nagase H, 1997), a serine proteinase, which can also degrade articular cartilage (Moore AR, 1999). Furthermore, NE activates latent stromelysin-1 (Nagase H, 1997), an important MMP involved in joint destruction (Van Meurs J, 1999). Neutrophil elastase is inhibited principally by the major serum protein α 1-proteinase inhibitor (α1-PI) (Carell RW, 1986). Therefore α1-PI is potentially an important inhibitor of rheumatoid joint damage.

The reactive centre of α1-PI is crucial to its ability to inhibit NE (Taggart C, 2000). This reactive site contains a methionine group that is rapidly oxidised and therefore inactivated by potent antioxidants such as hypochlorous acid (reviewed by Evans M, 1994). Hypochlorous acid can be produced by a neutrophil enzyme: myeloperoxidase (MPO) in

combination with hydrogen peroxide (H_2O_2) via the MPO - H_2O_2 -chloride system (Weiss SJ,

1987). It has been demonstrated that oxidation of α1-PI by this system promotes binding to

immunoglobulin A and the formation of IgA-α1 PI complexes (Scott LJ, 1999). These

complexes form as a result of disulphide binding of α1-PI to the penultimate C-terminal

cysteine residue of the IgA C-α3 domain which normally binds J-chain (Scott LJ, 1999).

Raised levels of the IgA-α1 PI complex correlated significantly with anatomical

progression of RA (Lacki JK, 1995). The authors could not exclude the possibility that the

constant high level of IgA-α1 PI may cause worsening in bone erosions. A good correlation

has been established between IgA and IgM RF (r = 0.61, p < 0.01), in a group of Polish RA

patients (Lacki, 1994).

Seropositive, erosive and nodular RA cases have significantly higher serum IgA-α1

PI complex levels than both seronegative RA cases and healthy controls (Scott LJ, 1998).

Importantly RA cases with a high serum IgA-α1 PI complex level have a greatly reduced

inhibitory capacity to NE (Scott LJ, 1999). For the reasons outlined above, I believe that

such individuals with high serum IgA-α1 PI complex levels may be more susceptible to

erosive joint disease.

Cigarette smoke is abundant in free radicals and can inactivate α1-PI (reviewed by

Evans, 1994). Both cigarette smoking (Saag KG, 1997; Wolfe F, 2000; Mastottir B, 2000)

and high serum levels of IgA-α1PI complex (Scott LJ, 1998) are associated with seropositive,

erosive and nodular RA. Accordingly I hypothesise that cigarette smoking is associated with

the high serum levels of IgA-α1PI complex observed in RA. To test this hypothesis I have

compared serum levels of IgA-α1PI complexes in smoking and non- smoking healthy

individuals and RA cases.

Methods

Study design

This study was a case-control study. Patients and controls gave informed consent and participated in the study. The ethics committees in each participating centre approved the study.

Patient and control population

Adult outpatients with RA (age range 19-88) all fulfilling the ARA criteria for RA (Arnett FC, 1988) and with a clinical diagnosis of RA attending two hospitals in Merseyside, St.Helens Hospital and University Hospital Aintree were recruited for the study. The 231 RA patients were selected to represent a cross-section of rheumatoid disease to test my hypothesis and therefore approximately equal numbers of smokers, ex-smokers and life long non-smokers were recruited. The patients' age and age of disease onset were recorded. These patients were a different cohort to those studied in chapters two and three. A case note examination of the RA cases was undertaken to determine both RF status and for radiological evidence for erosive joint disease. Seropositive patients were defined as seropositive if previously the rheumatoid latex test had been positive at a titer of 1:40 or greater. RA patients with active infection or chronic infective processes such as bronchiectasis were excluded from the study. The demographic and clinical characteristics of the RA patients and controls are shown in Table 8. The 83 control cases were recruited from healthy hospital workers.

Smoking History

A comprehensive past and current smoking history was taken from time of inclusion to the study. Cigarette consumption was quantified in pack years (PY). One PY is equivalent

to 20 cigarettes smoked per day for one year. A current smoker was taken to be a smoker of at least 1 cigarette per day for 1 year. An ex-smoker was defined as an individual who had stopped smoking for at least 3 months.

IgA-α1PI complex levels and laboratory assessments.

A single 10ml venous sample was taken from each case. The blood was centrifuged for five minutes and the plasma stored at -70^0C. The IgA-α1PI complex levels (arbitrary units, au) were determined using a sandwich ELISA. The methodology has been described previously (Scott LJ, 1998). A reference plasma sample from an individual with high levels of IgA-α1PI complex was used to obtain a standard curve from which arbitrary units (a.u.) were obtained. The erythrocyte sedimentation rate (ESR) was measured in the RA cases only.

Statistical analysis

The relationships between IgA-α1PI complex levels and smoking status, erosive RA and RA disease activity were examined by multiple regression analyses with correction for the independent variables, age, gender, and disease duration where appropriate. In cases where variables were not normally distributed they were transformed to normality by using the log values. Multivariate logistic regression analysis was used to determine the variables most closely associated with erosive RA. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v.6.0.4).

Results

Influence of smoking on IgA-α1PI complex levels in RA patients and healthy controls

RA patients who had ever smoked had significantly higher IgA-α1PI complex levels than those who had never smoked (p = 0.01) (Table 9). Division of ever smokers into past

and current smokers revealed that the increased complex levels were associated only with the current smokers (p = 0.0001). The RA past smokers had similar complex levels to the RA group that had never smoked. Similar results were found in the healthy control population, the highest complex levels again being found in current smokers (p = 0.003). No significant differences in complex levels were found between non-smoking RA patients and non-smoking controls, or between RA patients and controls that smoked. Correction for age made no difference to these analyses.

I found no relationship between number of cigarettes smoked per day and complex levels in the RA current smokers. However, in all patients who had ever smoked a weak association was found between pack years smoked and complex levels (p = 0.05, after correction for sex and disease duration). No association was found in the healthy control group.

Relationship between IgA-α1PI complex levels and seropositivity in RA patients

Complex levels in RF+ RA patients (n = 169) were significantly higher than in RF- patients (n = 62) (16.1 v 8.8 a.u., p < 0.0001, after correction for sex and disease duration). Multiple regression analysis demonstrated that seropositivity was associated with higher complex levels independently of current smoking after correction for sex and disease duration. In this model the association of complex levels with RF positivity was stronger (p = 0.0001) than that with current smoking (p = 0.013).

Relationship between IgA-α1PI complex levels and ESR levels

I found a significant association between IgA-α1PI complex and ESR levels in the total RA population (p = 0.004, after correction for sex and disease duration by multiple regression analysis). This association remained significant after further correction for current smoking and seropositivity (Table 10).

Relationship between IgA-α1PI complex levels and erosive disease

Complex levels in patients with erosive disease (n = 151) were significantly higher than in patients with non-erosive disease (n = 80) (15.9 v 10.5 a.u., p = 0.001 after correction for sex and disease duration). However, significance was lost in a multivariate logistic regression analysis that also included current smoking and RF positivity as independent variables (Table 11).

Discussion

This study has shown that RA patients who are current smokers have significantly higher IgA-α1PI complex levels than non-smoking RA patients. However, I have also found that healthy individuals who smoke have elevated IgA-α1PI complex levels that are comparable with those found in RA patients who smoke. This would suggest that high IgA-α1PI complex levels in RA smokers do not occur as a result of RA *per se*, but are produced also as a result of cigarette smoking. I also found that a marker of RA disease activity (ESR) was associated with IgA-α1PI serum complex levels independently of current smoking. These data suggest that high IgA-α1PI serum complex levels can be generated either as a result of current smoking, or by an active disease process in RA patients. I hypothesise that this complex formation is induced through oxidative stress that is generated either by smoking, or the rheumatoid disease process itself.

I confirmed previous findings of increased IgA-α1PI complex levels in RF+ patients (Scott LJ, 1998). Additionally I found that the association of complex levels with seropositivity was independent of current smoking. This does not appear to be due to any effect of RF on the IgA-α1PI assay since it has previously been shown that RF does not interfere with this assay (Scott LJ, 1998). Although both RF and IgA-α1PI are associated with current smoking they appear to be produced independently, and they demonstrate differences in their dependence on overall smoking history. For example, there was only a very weak association between pack years smoked and complex levels in the RA group. This is in contrast to studies that have observed a strong association between pack years smoked, increased RF concentration and joint damage (Saag KG, 1997; Wolfe F, 2000; Masdottir B, 2000). Furthermore, RF production, unlike high serum levels of IgA-α1PI complex, may persist in heavy ex-smokers. Former heavy smoking RA patients have been observed to have similar RF levels to current heavy smokers (Wolfe F, 2000) and a recent study observed that

RF persisted despite the cessation of smoking in healthy individuals (Heliovaara M, 2000). My data suggest that the continual generation of IgA-α1PI complexes may depend on continuing oxidative stress and that high serum levels of these complexes return to normal once the source of the oxidative stress (i.e. smoking) has been stopped.

The mechanisms by which current smokers with RA are more likely to develop erosive disease than non-smokers with RA are not known. It has been recently demonstrated that smoking can induce both erosive and nodular rheumatoid disease independently of rheumatoid factor (Wolfe F, 2000). These data confirm an independent association of RF and current smoking with erosive disease, although RF has by far the strongest association. I also found that IgA-α1PI complex levels were higher in patients with erosive disease, although significance was lost after correction for RF and current smoking. Nonetheless, it is possible that the effects of smoking may be mediated in part through increased IgA-α1 PI complex formation. Cigarette smoke contains an abundance of free radicals and has been observed to oxidise and inhibit the function of α1PI (Evans M, 1994) The high complex levels observed in some RA patients who currently smoke may partly explain the association of current smoking with the development of erosive RA. In these patients significant joint destruction could possibly occur through a process involving increased neutrophil elastase activity. Neutrophil elastase may directly degrade cartilage or indirectly upregulate MMP activity by inhibition of a tissue inhibitor of MMPs (TIMP 1).

This study did not directly address whether a reduced capacity to inhibit NE predisposes to rheumatoid joint damage. Some erosive RA patients who were currently smoking did not have raised complex levels. Thus, in these patients joint damage does not appear to be associated with oxidation of α1-PI, although oxidative stress may still play a role (Shingu, 1994). Free radicals may activate MMPs, cysteine and other serine proteinases and cause direct damage to cartilage (Mantle D, 1999). Equally, free radical mediated

alteration of IgG may stimulate the formation of immune complexes with RF antibody, thereby promoting tissue damage during rheumatoid inflammation (Lunec J, 1988; Swaak AJ, 1989).

In conclusion, levels of serum IgA-α1PI complex are significantly raised in both healthy smokers and RA smokers. Serum IgA-α1PI complex levels correlate with ESR levels independently of current smoking or RF positivity in RA. These data suggest that high IgA-α1PI complex levels can be generated either as a result of current smoking, or by an active disease process in RA patients. Further studies are needed to determine the prognosis of RA cases with raised levels of serum IgA-α1PI complexes and the possible genetic factors predisposing these individuals to increased production of IgA-α1PI complexes.

Table 8. Demographic and clinical characteristics of RA patients (n =231) and controls (n = 83)

	RA			Controls		
		n	%		n	%
Median age, yrs (range)	61.0 (19 -88)			44.0 (19-75)		
Age of onset, yrs (range)	49.0 (18 – 82)			NA		
Disease duration, yrs (range)	12.0 (1 - 52)			NA		
Median ESR, mm/hr (range)	31 (5-126)			NA		
Female		173	75		60	72
Current smoker		75	33		45	54
Ex-smoker		72	31		9	11
Never smoked		84	36		29	35
Rheumatoid factor positive		169	73		NA	
Erosive disease		151	65		NA	

Table 9. Influence of smoking on IgA-α1PI levels (mean ± SD) in RA patients and healthy controls

	Smoking status			
	Never	Ever	Past	Current
RA	n = 84	n = 147	n = 72	n = 75
	11.9(16.1)	15.1(16.2)*	12.8(13.2)	17.4(18.4) †
Controls	n = 29	n = 54	n = 9	n = 45
	11.5(5.9)	17.6(10.3)‡	11.8(2.9)	18.8(10.9)#

Multiple regression analyses with complex level as the dependent variable. The p values represents the significance of each smoking variable compared with never smoking.

* p = 0.01 (corrected for sex and disease duration).

† p = 0.0001 (corrected for sex and disease duration)

‡ p = 0.002 (corrected for sex).

p = 0.003 (corrected for sex).

Table 10. Multiple regression analysis to investigate the association of ESR, seropositivity and current smoking with IgA-α1PI levels in RA patients.

Independent Variable	Regression Coefficient	Standard error	p value
RF (+/-)	0.424	0.134	0.002
Current smoking	0.366	0.125	0.003
log ESR	0.182	0.072	0.011

Multiple regression model with log IgA-α1PI complex level as the dependent variable. The model was corrected for sex and disease duration by including these covariates with the independent variables. F-ratio 7.90, $p < 0.0001$.

Table 11. Multivariable determinants of erosive disease in RA.

Variable	Regression Coefficient	Standard error	p value
Current smoker	0.836	0.422	0.043
RF (+/-)	2.237	0.423	<0.0001
log IgA-α1PI	0.336	0.227	0.14
Duration	0.141	0.029	<0.0001
Female Sex	0.852	0.422	0.044

Chapter 6

Smoking and disease severity in RA: Influence of polymorphism at GSTM1 loci.

Introduction

A number of studies have suggested that smoking is a risk factor in susceptibility to RA (Vessey, 1987; Hernandez-Avila MH, 1990; Heliovaara M, 1993; Voight LF, 1994; Silman AJ, 1996; Symmons DPM, 1997; Karlson EW, 1999; Uhlig T, 1999; Hutchinson D, 2001; Olsson AR, 2001). However, fewer studies have investigated the impact of smoking on disease outcome. Four recent studies have suggested that heavy smoking may influence overall severity or extra-articular rheumatoid manifestations such as rheumatoid nodules (Saag KG, 1997; Wolfe F, 2000; Masdottir B, 2000; Harrison BJ, 2002). All of these studies reported a relationship between smoking and RF positivity and nodularity. Heavy smoking was associated with increased joint damage (Saag KG, 1997; Wolfe F, 2000; Masdottir B, 2000). Harrison 2002 did not reproduce these findings, in a cohort of polyarthritis cases with a follow up period of three years.

Rheumatoid factor is certainly strongly associated with smoking in RA. Wolfe F, 2000 observed RF levels to be correlated with pack years smoked and years of smoking. Another recent study showed that the levels of IgA RF and IgM RF, but not IgG RF were associated with number of pack years smoked (Masdottir B, 2000).

The mechanism by which cigarette smoking increases RF production is currently unknown. No research to date has addressed this important question. I postulate that genes associated with detoxification or activation of chemicals in tobacco smoke will be important. In this respect a previous finding that increased severity in RA is associated with a null polymorphism at the glutathione S-transferase GSTM1 locus is of particular interest (Mattey DL, 1999).

The GSTs are a widely expressed group of enzymes that catalyse the detoxification of xenobiotics via glutathione conjugation, and are also believed to play an important role in detoxifying products generated by the activity of reactive oxygen species (ROS). There is

evidence that some allelic variants are associated with differences in detoxification efficiency, and various cancer studies have suggested that polymorphism in GSTM1 may influence the ability to detoxify chemicals in cigarette smoke (Rebbeck TR,1997;Seidergard J,1990;Chenevix-Trench,1995).

Two recent studies have suggested that GST M1 null individuals with Sjogrens syndrome have a significantly increased risk of developing particular antibodies (Ro antibodies) (Ollier W, 1996;Morinobu A, 1999).A synergistic interaction has been observed between smoking and GSTM1 null on the risk of coronary heart disease (Li R, 2000)

In this study I wished to test the hypothesis that the impact of smoking on disease outcome is associated with polymorphism in the GSTM1 gene. The hypothesis was developed *a priori* with GSTM1 null being considered as the putative high risk genotype. It is noteworthy that the study could only be carried out on female RA patients since most of the male patients (~90%) in the population studied had been smokers. Thus, in the male population it was not possible to test for statistically significant differences between males who had smoked and those who had not.

Materials and Methods

Patients.

The association between GSTM1 genotypes, smoking and disease severity was studied in 164 unrelated female RA patients resident in North Staffordshire. All patients were Northern European Caucasians. The characteristics of these patients are shown in Table 12. The patients fulfilled the 1987 ARA criteria (Arnett FC, 1988), and were recruited in a clinic established to examine the effects of disease modifying anti-rheumatic drugs. These included methotrexate, sulphasalazine, gold, hydroxychloroquine, and D-penicillamine.

About 6.5% of patients were being treated with corticosteroids. Therapy was administered as clinically indicated.

All patients had been reviewed annually for at least 5 years and their disease extensively characterised. Outcome measures were recorded at final review and consisted of assessment of functional status using the Health Assessment Questionnaire (HAQ) (Fries JF, 1980) and radiographic outcome, obtained by scoring X-rays of the hands and feet using the standard radiographs of Larsen (Larsen A, 1977). Rheumatoid factor levels were measured using nephelometry and reported in International Units (IU). A level greater than 60 IU/ml was considered to be positive (Wolfe F, 1998)

The Carstairs deprivation index (Carstairs V, 1991 and 1995) was used to categorise the socio-economic status of the patients. Carstairs scores were obtained from the 1991 census small-area statistics for the United Kingdom (UK) and assigned to each patient based on their enumeration district of residence identified from the postcode address. The index is based on a composite of four variables; male unemployment, social class of head of household, overcrowding and access to a car. The Carstairs scores in the RA population for this study ranged from -4.6 (least deprived) to +5.81 (most deprived).

Smoking history

A history of current and past smoking was obtained from each patient. Patients were initially classified by whether they had ever or never smoked. Ever smokers were those that had smoked at least 1 cigarette a day for 1 year or more, but were not necessarily smoking at the time of the study. All patients who had ever smoked had started smoking before the onset of RA. Those who had ever smoked were further categorized into past and current smokers. Past smokers were those that had stopped smoking at least 3 months before entry into the

study. The extent of smoking was quantified in pack years (PY). One PY is equivalent to 20 cigarettes per day, every day for one year.

GST typing.

Leukocyte DNA was extracted from blood samples collected in EDTA. GSTM1 genotypes were defined using a PCR assay that identifies the GSTM1*0, GSTM1* A and GSTM1*B alleles (Elexpuru-Camiruaga J, 1995). Patients were classified into GSTM1 expressing (GSTM1-1) and non-expressing (GSTM1-0) individuals.

Statistical analysis.

The association of disease severity measures with GSTM1 genotypes and smoking was assessed using multiple regression analyses with adjustment for the independent variables, age, and disease duration where appropriate. The influence of rheumatoid factor (RF) and the Carstairs deprivation index were also examined by inclusion as independent variables in some of the regression models. Additional analyses were carried out on the patient population stratified by GSTM1 status. In some analyses we also examined the effect of interaction between variables using multiple regression models that contained the interaction term as well as the corresponding main effects. Where appropriate, correction for potential multiple testing errors was performed using Holm's procedure (Holm S, 1979) All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v.6.0.4), or the PEPI software package (v2.0) or epidemiological analysis (Gahlinger,1995).

Results

Frequency and extent of smoking in female RA patients

About half (51.2%) of the patients had ever smoked (Table 13). The number of smokers had dropped to 29.9% by the time of the study. In this group the mean duration of smoking and number of pack years were 38.9 and 29.3 years respectively (Table 14), which were significantly greater than in past smokers (23.5 and 18.5 years respectively). There was a significantly higher frequency of seropositive (RF+) disease in current smokers compared with those who had never smoked.

Relationship between smoking and disease severity in female RA.

The mean Larsen score of patients who had ever smoked was significantly higher than those who had never smoked (p_c = 0.01 after correction for age and disease duration) (Table 15). The trend was similar in current smokers (p_c = 0.05). The highest mean Larsen score was found in past smokers (112.2), and this was significantly higher than in those who had never smoked (p_c = 0.006). Using multiple regression analyses corrected for age and disease duration we found that in ever smokers there was no association between Larsen score and the number of pack years smoked (p = 0.43). HAQ scores showed a similar trend to Larsen scores, with ever, past or current smokers having significantly increased HAQ scores compared to those who had never smoked (Table 15). Again, no association was found between HAQ score and the number of pack years smoked (p = 0.68).

Influence of GSTM1 polymorphism.

Comparison of Larsen scores in GSTM1 null and GSTM1 expressing patients by multiple regression analysis revealed a similar trend to that reported previously (Mattey DL, 1999) although this did not achieve significance after correction for age and disease duration (99.4 v 86.8, p= 0.1). There was no significant difference in HAQ scores (1.57 v 1.59).

In order to investigate whether polymorphism in the GSTM1 gene influenced the disease response to smoking the patients were stratified by GSTM1 status (expressing versus non-expressing individuals) as well as never or ever smoking. GSTM1 null patients who had ever smoked had significantly higher Larsen (p_c = 0.012) and HAQ scores (p_c = 0.003) than GSTM1 null patients who had never smoked (Table 16). Radiographic outcome in GSTM1 null patients who had smoked was also worse than in GSTM1 expressing individuals who had smoked (p_c = 0.05), although there was no significant difference in HAQ score. In GSTM1 expressing patients there were no significant differences in Larsen or HAQ scores between ever smokers and non-smokers.

Association between GSTM1 polymorphism, smoking and rheumatoid factor production

There was a significant association between ever or current smoking and RF positivity (p = 0.006 and 0.004 respectively), after correction for age and disease duration (Table 13). In all smokers the RF level was also associated with the number of years of smoking (p = 0.02), but not with the number of pack years (p = 0.17). However RF positivity in smokers did show an association with number of pack years (p = 0.03). The reason for this discrepancy is not clear, but suggests that RF positivity provides a more robust correlate than measurement of RF levels at a single point in time.

It was postulated that the development of more severe disease in GSTM1 null patients who smoked might be associated with a difference in the production of RF by these patients. The association between RF status and smoking in patients stratified by GSTM1 genotype was examined. Using logistic regression analysis adjusted for age and disease duration we found no significant difference in RF status between ever and never smoking (67.7 vs 51.4%, OR 1.9, p = 0.2) in GSTM1-1 expressing patients. However, in GSTM1 null patients there was a

difference in RF status between ever and never smoking (75.5 vs 51.2%, OR 3.1, p = 0.01).

Division into past and current smokers revealed that this difference was specifically due to a

difference between current and never smoking in these patients (83.9 vs 51.2%, OR = 5.1, p =

0.006) (Table 17). No significant difference in RF status was found between current and

never smoking in GSTM1-1 patients (64.7 vs 51.4%, OR = 1.7, p = 0.4).

With regard to the amount of RF production, there was a significant association between

current smoking and RF levels in GSTM1 null patients (p = 0.002), but no association in

GSTM1-1 patients (p = 0.4). Confirmation of the relationship between current smoking and

RF status in GSTM1 null (p = 0.005) but not GSTM1-1 patients (p = 0.3) was obtained from

a separate cohort of RA patients (n = 134) with early disease (median duration = 1 year) (data

not shown).

Multiple regression models which included RF (+/-) as well as GSTM1 null + ever

smoking as independent variables showed that RF status had the strongest association with

Larsen score after correction for age and disease duration. (Table 18). Nonetheless, GSTM1

null + ever smoking remained associated with radiographic outcome after correction for RF,

although the significance levels were greatly reduced. A similar observation was found for

the HAQ score, although the association with RF status was weaker, and age had a more

significant effect than disease duration (Table 19). These data suggest that the association of

GSTM1 null + ever smoking with disease severity is not due solely to RF status. However,

the possible contribution of other, as yet undiscovered, confounder or modifier variables to

the association of this combination variable with the outcome measures cannot be excluded.

Influence of socio-economic status

It was investigated whether socio-economic deprivation had any effect on disease

severity. There was no association between the Carstairs index and radiographic outcome in

univariate or multivariate models (data not shown). In contrast, the HAQ score was

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associated (p = 0.015) with the Carstairs index in models corrected for age and disease duration only, but lost significance in mutivariate models containing GSTM1 null + ever smoking and RF status (Table 19). Removal of GSTM1 null + ever smoking from the latter model resulted in a weakly significant association between the Carstairs and HAQ scores (p = 0.04).

Discussion

In a well-characterised group of female RA patients it has been demonstrated that a history of cigarette smoking is associated with more severe disease. The observations of other studies that smokers were more likely to be RF positive than non-smokers, and that the number of years smoked was associated with RF levels (Saag KG,1997;Wolfe F,2000) was confirmed. Furthermore the relationship between smoking and disease severity in these patients is associated with polymorphism at the GSTM1 locus.

These data suggest that the risk of developing severe disease in female patients is increased in individuals with the GSTM1 null polymorphism who have also smoked. The difference between these patients and GSTM1 null patients who had never smoked was highly significant, although significance levels were reduced after correction for RF status. In contrast there was no significant difference in disease severity or RF levels between smokers and non-smokers demonstrating the functional GSTM1-1 phenotype. These data suggest that deletion of the GSTM1 gene only appears to influence RA severity in smokers. Accordingly the difference in joint damage between patients who have never or ever smoked appears to be accentuated in individuals with the GSTM1 null polymorphism. This would not appear solely to be as a result of increased RF production as this process was independent of RF. However, this finding must be interpreted with a degree of caution. Rheumatoid factor levels may fluctuate in the course of rheumatoid disease, whereas a joint score is a measure of a cumulative disease process over many years. Therefore a "one off" RF represents only a snap shot in time and may not represent previous RF levels during the previous disease process. Nevertheless Wolfe, 2000 also observed that cigarette smoking was associated with rheumatoid joint damage independent of RF.

Potential mechanisms by which smoking in combination with the GST M1 polymorphism increases rheumatoid joint damage independently of RF include 1) Increased

oxidative damage to α1-PI with a subsequent increased risk of developing rheumatoid erosions (see chapter 5). GST M1 is known to play a role in the detoxification of reactive oxidative species and may therefore be relevant to this process. 2) Increased activity of MMP 1. MMP 1 is an important cause of joint erosions independent of the acute phase response (Cunnane G, 2001). GST M1 inhibits benzo(α)pyrene and other polycyclic hydrocarbons (Rebbeck TR, 1997). Benzo(α)pyrene is an important cause of mutations to P53, an important protein in the pathogenesis of lung and bladder cancer (Sundberg K, 2002;Brockmoller J, 1996). A recent pooled analysis and meta-analysis of GST M1 and bladder cancer observed an additive interaction between GST M1 null and smoking (additive interaction=0.45,95%CI:-0.03,0.93) (Sundberg K, 2002). Recently studies have demonstrated that P53 inhibits MMP 1 activity and that mutagenesis to P53 stops this inhibitory process and subsequently there is an up-regulation of MMP-1 (Sun Y, 1999).

The main finding of this study was that the association between current smoking and RF status was significant only in GSTM1 null patients, where those who smoked had the highest levels of RF. The correlation between smoking and RF has made it difficult to determine the independent predictive value of each of these factors in relation to disease severity. From this data it is possible to speculate that lack of the GSTM1 enzyme in smokers may promote increased RF production through a failure to detoxify smoke-derived chemicals (or their by-products) that have the potential to damage IgG.

Previous studies have suggested that free radical mediated alteration of IgG may stimulate the formation of immune complexes with RF antibody, thereby promoting tissue damage during rheumatoid inflammation (Lunec J,1988;Swaak AJ,1989).

Saag et al, 1997 suggested that smoking may be more important in the initiation of erosive disease than in the perpetuation of the disease process. The lack of an obvious dose-related effect of smoking on the amount of radiographic damage in this study may add some

weight to this idea. Although these data also suggest that if smoking is involved in the initiation process it leads to more severe disease than it does in patients where it is not involved (particularly if the patients are GSTM1 null).

It might have been expected that increased exposure to cigarette smoke would have led to increased damage and more severe disease. However, the outcome in past smokers was as severe as in those who continued to smoke, even though the number of years smoked and pack years was significantly lower in the former. Nonetheless, the mean duration and amount of smoking in the past smokers was relatively high, and compared with other studies was close to the levels considered to represent a history of heavy smoking (Karlson EW,1999;Saag KG,1997;Wolfe F,2000). There is evidence to suggest that individuals who smoked in the past continue to produce RF, even after cessation of smoking (Wolfe F,2000;Heliovaara M,2000) Thus, tissue damage mediated by RF complexes may continue long after the initial stimulus for RF production has been removed.

An alternative explanation for the lack of a dose-related effect could be that some other factor(s) in female smokers predisposes them to develop more severe RA, especially if they are GSTM1 null. Potential confounders such as body mass index (BMI), estrogen levels, oral contraceptive use, or alcohol intake may be important, but without available data on these variables we were unable to test their effect in our study. However, a recent report from Karlson et al, 1999 suggested that BMI and oral contraceptive use were not significant confounders in the association between smoking and RA susceptibility. Other possible confounders such as diet, or exposure to environmental toxins may also have some influence but would be difficult to quantify.

The observed associations do not appear to be related directly to social deprivation since adjustment for socio-economic status using the Carstairs index did not affect the association between smoking and disease severity in these patients. This is consistent with

the findings of Saag et al, 1997 who found that the association of smoking with radiographic changes or RF positivity did not vary with any socio-demographic factors tested. There was no association between the Carstairs index and radiographic outcome with or without inclusion of GSTM1 null + smoking in the regression models, although a relationship between functional outcome and the Carstairs index after exclusion of GSTM1 null + smoking was observed. This is in agreement with previous studies that showed a relationship between functional outcome and social deprivation (McEntegart A,1997;ERAS Study Group,2000). However, these studies did not investigate the influence of smoking, and these data suggest that the relationship between social deprivation and functional outcome is at least partly due to the influence of smoking. It needs to be born in mind that the HAQ score may not be a specific indicator of the functional impact of RA severity per se. This may be particularly true among patients who smoke, given that smoking is likely to have adverse effects on general health and function.

One limitation of this study is its cross-sectional nature, and the difficulty in establishing cause and effect. It has been argued that such studies are subject to misclassification of smoking status, and that patients with very mild disease (who no longer seek medical care) or very severe disease (who may have died) are underrepresented (Harrison BJ, 2001). While prospective studies may overcome some of these difficulties, they tend to be of relatively short duration, and may not properly address the long-term impact of smoking on disease severity. They may also suffer from the difficulty of classifying RA in patients with early inflammatory polyarthritis. It is unlikely that misclassification of smoking status is a problem in our current study, especially as no difference in disease outcome between past and current smokers was found. However, this study is clearly limited to women with well established RA in one particular geographic area,

and further studies are needed on different populations to establish the validity of these findings.

Table 12. Demographic and clinical characteristics of female RA patients (n =164)

		n	%
Median age, yrs (range)	57.0 (32 -79)		
Median age of onset, yrs (range)	46.0 (19 - 73)		
Disease duration, yrs (range)	11.0 (5 - 30)		
Rheumatoid factor positive		102	62.2
Nodular disease		31	18.9
Erosive disease		148	90.2
Previous joint surgery		54	32.9

Table 13. Frequency (%) of cigarette smoking and seropositivity in 164 female RA patients

Smoking status	n	% patients	% RF +
Never	80	48.8	51.3
Ever	84	51.2	72.6*
Past	35	21.3	65.7
Current	49	29.9	77.5†

Comparisons for RF positivity were made with patients who had never smoked (adjusted for age and disease duration).

* OR = 2.5 (95% CI 1.2 - 5.0) p = 0.006,

† OR = 3.2 (95% CI 1.3 - 7.8) p = 0.004

Table 14. Extent of cigarette smoking in female RA patients

Smoking status	n	Yrs smoked (mean, SD)	Pack yrs (mean, SD)
Ever	84	32.5(13.3)	24.8 (16.1)
Past	35	23.5(13.4)	18.5 (17.5)
Current	49	38.9 (9.7)*	29.3 (14.4) †

* p < 0.0001 (compared with past smoking)

† p < 0.001 (compared with past smoking)

Analyses were corrected for age and disease duration.

Table 15. Relationship between smoking and radiographic or functional outcome in female RA patients. The mean scores and SD are shown.

Outcome measure	Smoking status			
	Never	Ever	Past	Current
	(n=80)	(n=84)	(35)	(n=49)
Larsen score	83.1(47.2)	104.7(49.9)*	112.2(47.7)†	99.3(51)#
HAQ score	1.39(0.8)	1.77(0.8) †	1.86(0.7)‡	1.71(0.8)

Multiple regression analyses (compared with never smoking, adjusted for age and disease duration): P values are shown uncorrected and corrected for multiple comparisons by Holm's procedure.

† $p = 0.002$, $p_c = 0.006$

* $p = 0.005$, $p_c = 0.01$

‡ $p = 0.009$, $p_c = 0.018$

¶ $p = 0.02$, $p_c = 0.02$

$p = 0.05$, $p_c = 0.05$

Table 16. Relationship between ever smoking and radiographic or functional outcome (mean ± SD) in female RA patients stratified by GSTM1 status.

	n	Never	n	Ever
		Smoking status		
Larsen score				
GSTM1-0	43	82.8(49.7)	53	128(53.3)*
GSTM1-1	37	83.5(44.9)	31	90.8(40.9)
HAQ score				
GSTM1-0	41	1.28(0.8)	50	1.81(0.8)†
GSTM1-1	37	1.50(0.9)	31	1.71(0.7)

Multiple regression analysis (adjusted for age and disease duration): P values are shown uncorrected and corrected for multiple comparisons by Holm's procedure.

* p = 0.006 (compared with GSTM1-0 + never smoking), p_c = 0.012

* p = 0.05 (compared with GSTM1-1 + ever smoking), p_c = 0.05

† p = 0.001 (compared with GSTM1-0 + never smoking), p_c = 0.003

Table 17. Relationship between GSTM1 status, current smoking and RF production in female RA patients

Patients	RF+ frequency				Rheumatoid factor levels (IU)	
	current smokers		never smoked		current smokers	never smoked
	n	%	n	%		
GSTM1-0	26/31	83.9*	19/37	51.2	284.7(302.9)†	69.8(137.8)
GSTM1-1	11/17	64.7	21/41	51.4	175.4(289.5)	74.7(138.1)

* Odds ratio 5.1 (95% CI 1.6 - 15.3), p = 0.006 (compared with never smoked)

† p = 0.002 (compared with never smoked)

Comparisons corrected for age and disease duration.

Table 18. Multivariable determinants of Larsen score in female RA

Variable	Regression coefficient	Standard error	p
Constant	9.993	16.387	
RF	19.092	6.162	0.002
GSTM1-0 + ever smoke	17.097	7.016	0.033
Disease duration	3.813	0.471	<0.0001
Age	0.379	0.264	0.151

Multiple regression analysis with Larsen score as the dependent variable. The p values for RF and GSTM1-0 + ever smoke represent the significance of each variable compared with individuals negative for that variable or combination of variables (i.e. RF+ versus RF-, GSTM1-0 + ever smoke versus the remainder). F value for the model = 24.56 (p<0.0001)

Table 19. Multivariable determinants of HAQ score in female RA.

Variable	Regression coefficient	Standard error	p
Constant	0.198	0.304	
GSTM1-0 + ever smoke	0.434	0.141	0.002
Carstairs score	0.040	0.023	0.08
Age	0.019	0.008	0.0002
RF	0.264	0.121	0.03
Disease duration	0.017	0.009	0.056

Multiple regression analysis with HAQ score as the dependent variable. The p values for RF and GSTM1-0 + ever smoke represent the significance of each variable compared with individuals negative for that variable or combination of variables. F value for the model = 9.88 (p<0.0001)

Chapter 7

Serum complexes of IgA-α1PI in RA: No evidence of an interaction with current cigarette smoking and polymorphism in the GST M1 gene.

Introduction.

There is likely to be a complex interplay between environmental and genetic factors in the development of RA. Cigarette smoking is an important trigger factor for the development of erosive RA (Saag KG, 1997; Wolfe F, 2000; Masdottir B, 2000; Mattey DL, 2002). RA individuals lacking the GST M1 gene (GST M1-0) have greater joint damage than RA individuals who express the GST M1 gene (GST M1-1) and this association is similar to and independent of the presence of the rheumatoid shared epitope (Mattey DL, 1999). Additionally I have observed that specifically GST M1-0 current cigarette smokers as opposed to GST M1-1 smokers and GST M1-0 non-smokers have greatly increased RF production and greatly increased joint damage. This represents an important genetic-environmental interaction in RA (Mattey DL, 2002).

However I have also observed that cigarette smoking worsens joint disease independent of RF in GST M1-0 individuals, suggesting other mechanisms may play a part in greater rheumatoid erosive damage in GST M1-0 smokers (Mattey DL, 2002). Wolfe has also observed cigarette induced joint damage independent of RF, although the GST M1 genotype was not studied (Wolfe F, 2000).

The prevailing hypothesis of the pathogenesis of rheumatoid erosions is an excess of matrix metalloproteinase activity within the joint stimulated by an exaggerated pro-inflammatory cytokine response (Goldring SR, 2000). However neutrophil elastase (NE) a serine proteinase may also have an important role (Moore AR, 1999). The natural inhibitor of NE, α1-PI, accounts for 90% of the NE inhibitory capacity of human serum (Ohlsson K, 1978). This inhibits NE by binding with its reactive site, which is susceptible to oxidation (Taggart C, 2000) and inactivation by free radical exposure (Carp H, 1979). Free radicals found in cigarette smoke cause oxidative damage to and decreased function of α1PI, reviewed in (Evans M, 1994). It has been demonstrated that oxidation of α1PI by the

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myeloperoxidase system promotes binding to immunoglobulin A and the formation of IgA-α1PI complexes and that individuals with raised serum IgA-α1PI complexes have a greatly diminished NE inhibitory capacity (Scott LJ, 1999). Furthermore serum levels of IgA-α1PI complexes are raised in erosive RA compared to other types of inflammatory arthritis (Scott LJ, 1998). I have recently demonstrated in chapter 5 that serum levels of IgA-α1PI complexes are raised as a consequence of cigarette smoking in addition to the disease process itself (Hutchinson D, 2002).

Glutathione plays an important role in the prevention of oxidative damage to α1-PI. (Gressier B, 1994). The GSTs are a widely expressed group of enzymes that catalyse the detoxification of xenobiotics via glutathione conjugation, and are also believed to play an important role in detoxifying products generated by the activity of reactive oxygen species (ROS) (Hayes JD, 2000). There is evidence that some allelic variants are associated with differences in detoxification efficiency, and various cancer studies have suggested that polymorphism in GSTM1 may influence the ability to detoxify chemicals in cigarette smoke (Rebbeck TR, 1997; Seidergard, 1990; Chenevix-Trench G, 1995).

My hypothesis is that GST M1 null current smokers are more likely to be susceptible to cigarette induced oxidative stress and therefore have raised serum levels of IgA-α1PI complex. To test this hypothesis I have measured the serum levels of IgA-α1PI complex in healthy hospital workers and RA patients and determined the GST M1 genotype in these individuals.

Methods

Study Design

This study was a case-control study. Patients and controls gave informed consent and participated in the study. The ethics committee approved this study for each of the participating centres.

Patient and Control Population

One hundred and fifty two outpatients with RA all fulfilling the ARA criteria for RA (Arnett FC, 1988) attending two hospitals (St.Helens Hospital and University Hospital Aintree) in the Merseyside region in the North-West of the UK were recruited for the study. The patients' age and age of disease onset were recorded. RA patients with active infection or chronic infective processes such as bronchiectasis were excluded from the study. Sixty control cases were recruited from healthy hospital workers.

The demographic and clinical characteristics of the RA patients and controls are shown in Table 20. A current smoker was taken to be a smoker of at least 1 cigarette per day, every day for 1 year.

IgA-α1 PI complex levels and laboratory assessments.

A single 10ml venous sample was taken from each case. The blood was centrifuged for five minutes and the plasma stored at -70^0C. The IgA-α1 PI complex levels (arbitrary units, au) were determined using a newly developed sandwich ELISA. A reference serum from an individual with high levels of IgA-α1 PI complex was used to obtain a standard curve from which arbitrary units (a.u.) were obtained. This is described in more detail in the methods section.

GST typing.

Leukocyte DNA was extracted from blood samples collected in EDTA. GSTM1 genotypes were defined using a PCR assay that identifies the GSTM1*0, GSTM1* A and GSTM1*B alleles. This is described in more detail in the methods section. Patients were classified into GSTM1 expressing (GSTM1-1) and non-expressing (GSTM1-0) individuals.

Statistical analysis

The relationship between IgA-α1PI complex levels and smoking and GST M1 status was examined by multiple regression analyses with correction for the independent variables, age, gender, and disease duration where appropriate. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v.6.0.4).

Results

Mean serum IgA-α1 PI complex levels in smoker and non-smokers

The mean serum IgA-α1 PI complex level was significantly raised in currently smoking control cases (n=28) compared to the non smoking control cases (n=32), 18.5 (11.0) a.u. vs. 11.5 (5.0) a.u., p<0.05. The mean serum IgA-α1 PI complex level were also significantly raised in RA smokers (n=114) compared to non-smoking RA cases (n=38), 14.2 (10.0) a.u. vs. 10.3 (13.0) a.u., p<0.05.

Mean serum IgA-α1 PI complex levels in GST M1-0 and GST M1-1 individuals

GSTM1-0 RA patients (n=72) had similar complex levels to GST M1-1 RA patients (n=80), 12.8 a.u. (18.7) vs. 10.1 a.u. (7.2), p>0.05. The GST M1-0 controls (n=27) had similar complex levels to GST M1-1 controls (n=33), 16.9 a.u. (12) vs. 15.6 (8.1), p>0.05.

Mean serum IgA-α1 PI complex levels in smoking GST M1-0 and GST M1-1 individuals

There was no interaction between current cigarette smoking and GST M1 status. Smoking GST M1-0 RA patients (n=54) had similar complex levels to smoking GST M1-1 patients (n=60), 14.4 a.u. (11) vs. 13.9 a.u. (9.5), p>0.05. Smoking GST M1-0 controls (n=9) had similar complex levels to smoking GST M1-1 controls (n=19), 20.5 a.u. (13.0) vs. 17.8 a.u. (10.0), p>0.05.

Mean serum IgA-α1 PI complex levels in never smoked GST M1-0 and GST M1-1 individuals

Considering RA patients and controls who had never smoked there was no significant difference in complex levels between GST M1-0 and GST M1-1 individuals. For never smoked RA GST M1-0 individuals (n=18) the mean complex levels were 12.3 a.u. (18.7) compared to 8.3 a.u. (7.2) for never smoked RA GST M1-1 individuals (n=20), P>0.05. For never smoked GST M1-0 control cases (n=18) the mean complex levels were 9.6 a.u. (4.2) compared to 13.9 a.u. (6.0) for never smoked GST M1-1 control cases (n=14), P>0.05. These data are summarised in tables 21 and 22.

Discussion.

As expected this study observed significantly higher serum IgA-α1PI complex levels in smokers compared to lifelong non-smokers. I did not, however, observe an interaction between GST M1-0 smokers and raised serum IgA-α1PI complex levels.

In RA GST M1-0 is only important in smokers and this group develop severe erosive disease (Chapter 6). These data presented here suggest that GST M1-0 is not associated with the development of raised serum IgA-α1PI complex levels. Accordingly I suggest that the RF independent mechanism of joint damage observed in GST M1-0 RA smokers in chapter 6 is

not as a result of oxidative damage to α1PI and the development of raised serum IgA-α1PI complex levels.

It is tempting to speculate that there may be three important subgroups of smokers who develop erosive disease. The underlying mechanisms in these three groups may be distinct both physiologically and genetically. It has been previously observed that GST M1-0 RA smokers have a four-fold increase in RF levels as opposed to non-smoking GST M1-0 RA patients (chapter 6). Rheumatoid factor is an important independent risk factor for the development of erosive disease (Combe B, 2001; Mattey DL, 2001; Kaltenhauser S, 2001). Smoking has also been observed to cause erosive joint damage independently of RF (Wolfe F, 2000). I have demonstrated that a raised serum IgA-α1PI complex level is a potential risk factor for erosive RA independent of RF (chapter 5). IgA-α1PI complex levels are not influenced by GST M1. Additionally GST M1 and smoking are associated with increased erosive disease independent of RF. It is therefore possible that smoking has at least three potential mechanisms of causing erosive RA disease, the RF mechanism largely GST M1-0 dependent (chapter 6), the IgA-α1PI mechanism (chapter 5) and the GST M1-0 RF independent mechanism described in chapter 6.

Considering the complex physiology of α1PI oxidation it is apparent that there are numerous candidate genes that may interact with cigarette smoke free radicals in such a way to produce a high oxidative stress that inhibits α1PI function.

Physiologically by far the most potent inhibitor of α1PI function is hypochlorous acid (HOCL) (Shock A, 1988). Activated neutrophils contain the enzyme myeloperoxidase, which uses hydrogen peroxide to oxidize chloride ions into a powerful oxidant that has been identified as HOCL (Weiss SJ, 1986). It has been previously demonstrated that oxidation of α1PI by the myeloperoxidase system promotes binding to immunoglobulin A and the formation of IgA-α1PI complexes (Scott LJ, 1999). Therefore the detoxifying systems that

prevent HOCL accumulation are likely to be important in determining the formation of IgA-α1PI complexes. These systems include both catalase and glutathione peroxidases which break down hydrogen peroxide, reviewed in (Mates M, 2000; Arthur JR, 2000). Interestingly severe RA is associated with low glutathione peroxidase activity, reviewed in (Tarp U, 1994).

Conversely superoxide dismutase catalyses the conversion of the superoxide radical to hydrogen peroxide and it has been recently demonstrated important differences in the severity of erosive RA between individuals with differing manganese superoxide dismutase polymorphisms (Mattey DL, 2000).

Glutathione is a ubiquitous antioxidant and has an important role in preventing oxidation of the α1-PI reactive site and this process is thought to be as a result of glutathione scavenging hydrogen peroxide (Gressier B, 1994). The role of the glutathione in RA has been reviewed in (Hassan MQ, 2000) and is suggested to be an important defense system in RA. Glutathione is involved in the elimination of reactive electrophiles and in conjugation to lipophilic compounds, to increase their solubility before they are excreted. These detoxyfication reactions are catalysed by glutathione s-transferases, which exist in at least ten isoenzyme forms with different substrate specificities. Therefore any one of a large number of GST genes other than GST M1 may be specifically involved in catalysing the conjugation of glutathione with hydrogen peroxide and therefore preventing oxidative damage to α1-PI as a result of diminished HOCL accumulation. The enzyme methionine-S-oxide reductase has been observed to restore functional activity to oxidised α1-PI (Morrison HM, 1986) and therefore polymorphisms of the methionine-S-oxide reductase gene may also be important.

There are potentially numerous candidate genes that may interact with cigarette smoke to cause α1-PI oxidation and therefore raised serum levels of IgA-α1PI complex levels. I feel these are likely to be important candidate genes for rheumatoid disease progression and the genetic screening of both healthy and RA individuals with raised serum

IgA-α1PI complex levels may help to determine genetic factors that determine a poor prognosis in RA.

Table 20. Demographic and clinical characteristics of RA patients (n =152) and controls (n

= 60)

	RA		Controls	
	n	%	n	%
Median age, yrs (SD) 60.5 (12.9)			44.3 (11.4)	
Median age of onset, yrs (SD) 49.6 (11.3)			NA	
Disease duration, yrs (SD) 10.9 (9.7)			NA	
Seropositive	106	70	NA	
Female	113	74	43	72
Current smoker	114	75	28	47
Never smoked	38	25	32	53

Table 21. Association between IgA-α1PI complex levels (mean ± SD) and GSTM1 status in all RA patients and controls.

	n	Complex level (a.u.)
RA patients		
GSTM1-0	72	12.8(18.7)
GSTM1-1	80	10.1(7.2)
Controls		
GSTM1-0	27	16.9(12.0)
GSTM1-1	33	15.6(8.1)

Differences are non-significant

Table 22. Relationship between current smoking and IgA-α1PI complex levels (mean a.u.±

SD) in RA patients and controls stratified by GSTM1 status.

Smoking status	Current		Never	
	n	Complex	n	Complex
RA patients				
GSTM1-0	54	14.4(11.0)	18	12.3(18.7)
GSTM1-1	60	13.9(9.5)	20	8.3(7.2.)
Controls				
GSTM1-0	9	20.5(13.0)	18	9.6(4.2)
GSTM1-1	19	17.8(10.0)	14	13.9(6.0)

No significant difference between GST M1-0 and GST M1-1 in smokers and non-smokers.

Chapter 8

Conclusions

This thesis is a collection of work regarding the relationship between RA and cigarette smoking. As described in detail in the introduction there is now considerable data to support a relationship between RA and cigarette smoking.

In Chapter 2 I have investigated the relationship between cumulative cigarette smoking (pack years smoked) and the development of RA requiring hospital follow up. I observed a dose dependant association between PY smoked and RA. A high cumulative exposure to cigarette smoke was associated strongly with RA. Secondly the RA patients with a family history of RA were significantly less likely to be current cigarette smokers at the time of disease onset and smoked significantly less PY than the RA group without a family history of RA. The inclusion of control cases from a different region of the North of England is unlikely to have introduced a bias as the control cases were matched for age, sex and social class which are the principal determinants of the prevalence of cigarette smoking (Living in Britain. The general household survey. Office of National Statistics, 1998). The control cases matched the RA patients with a family history of RA in terms of pack years smoked. This is important as the smoking history of the RA patients with a family history of RA is typical of that of the adult population aged 18-60 years for the region of the North West of England. Thirty nine per cent of the RA patients with a family history of RA were smoking at the time of disease onset, compared to thirty-nine per cent of adults (18-60) who are current smokers in our region of Merseyside (St. Helens) (Living in Britain. The general household survey. Office of National Statistics, 1998). The limitations of this study were that it was cross-sectional with a potential bias such that patients with more severe disease or with multi-medical problems are likely to be followed up more frequently than less severe cases. Therefore a study in a purely clinical setting will over represent more severe disease.

The collection of data in terms of pack years smoked was undertaken in a standard way and is a highly reproducible way of collecting a cigarette history.

Merseyside is a highly industrial, densely populated and socially deprived region of Northern England. This is in contrast to Norfolk, the region of England that forms the basis for the NOAR ARC epidemiology research. Norfolk consists principally of a rural, non-industrial and a relatively prosperous region of England. It is possible that an interaction between cigarette smoking and a socio-economic factor more prevalent in Merseyside may be of importance (Hutchinson et al, 2000). However I observed a similar association to that observed by Symmons (NOAR study) et al in a *community- based* study (Symmons DP, 1997) (OR 1.66, 95% CI 0.95-3.06). However the number of cigarettes smoked was not quantified in Symmons's study and no further direct comparisons can be made. My study demonstrates the need to quantify the number of cigarettes smoked when studying the relationship between cigarette smoking and its association with RA.

Since my study in chapter 3 was published there have been four publications on this subject. A Swedish study (Olsson et al, 2001) investigating co-morbidity and lifestyle in hospital outpatients based RA observed current and previous smoking were associated with increased risks for seropositive RA in both sexes. In both sexes there was a dose-response relationship found with number of tobacco pack years (p for trend <0.005 men and $p=0.029$ women.). The number of pack years smoked were banded <5, 5-9, 10-19, >20. Therefore high intensity smoking and modest smoking were grouped together. Current and previous smoking were associated with increased risks for RA in both sexes, and in men a dose-response relationship was found with number of tobacco pack years (p for trend <0.005). For men smoking > 20 pack years the risk for seropositive RA was increased significantly OR 3.4 (1.5-8.4) and for women OR 2.5 (0.9-6.7).

Criswell LA, 2002 studied whether cigarette smoking increases the risk of RA among postmenopausal women. A cohort of 31 336 women in Iowa who were aged 55 to 69 years in 1986 and who had no history of RA. Through 1997, 158 cases of RA were identified. Multivariable Cox proportional hazards regression was used to derive rate ratios (RRs) and 95% CIs for the association between cigarette smoking and RA. Compared with women who had never smoked, women who were current smokers (RR = 2.0; 95% CI: 1.3 to 2.9) were at increased risk of RA. Those who had quit 10 years or less before study baseline (RR = 1.8; 95% CI: 1.1 to 3.1) were at increased risk of RA, but women who had quit more than 10 years before baseline were not at increased risk (RR = 0.9; 95% CI: 0.5 to 2.6). Both the duration and intensity of smoking were associated with RA. Multivariable adjustments for age, marital status, occupation, body mass index, age at menopause, oral contraceptive use, hormone replacement therapy, alcohol use, and coffee consumption did not alter these results.

Additionally Masi, 2001 published his investigations regarding the relationship between RA and heavy cigarette smoking. A case-control study nested within a community based cohort (n=21061 adults) enrolled in 1974. Twenty years later 54 individuals were noted to develop RA. These were matched with four controls from the entry cohort and were matched for age, sex, and race (all white subjects). Heavy cigarette smoking (>30 cigarettes per day) was associated with RA (OR 21.5, 95% CI 1.9 to 122.5, p=0.005).

A very recent Finnish study (Krishnan E, 2003) studied 1095 patients with RA and 1530 control individuals Preliminary analyses revealed the presence of substantial statistical interaction between smoking and sex (P < 0.001). In separate multivariable analyses, past history of smoking was associated with increased risk for RA overall in men (odds ratio 2.0, 95% confidence interval 1.2-3.2) but not in women. Among men, this effect was seen only for seropositive RA. Interestingly in women, but not in men the smoking RA women had a significantly later age of onset than non-smoking RA women. I would contend that this

because of a pack-year susceptibility rather than a protective effect of smoking in female RA cases as suggested by the authors Krishnan E et al, 2003

I have observed a modest non-significant association between smoking and RA, in individuals who had smoked less than 30 PY. However, I observed that prolonged exposure to cigarette smoke resulted in a strikingly increased risk of developing RA for patients with a 40-50 pack year cigarette habit. This has implications for studies in countries where heavy cigarette smoking is extremely uncommon. An example of this is a large prospective study over 20 years in Finland designed to examine the relationship between cigarette smoking and the development of RA (Heliovaara M, 1993). The study observed no relationship between cigarette smoking and RA in Finnish women. However, only 2.5% of the women studied were current cigarette smokers of more than 15 cigarettes per day and few would be exposed to more than 30 PY of cigarettes.

It is noteworthy that the findings apply to the more severe RA cases seen in patients attending out-patients clinics, and are not necessarily true for milder, community-based RA. The majority of studies investigating the association between cigarette smoking and RA have been community-based studies (Uhlig T, 1999; Karlson EW, 1999; Symmons DP, 1997;Heliovaara M, 1993;Voight LF, 1994). The Merseyside population studied in chapter 3 had a very high prevalence of seropositive RA (90%). Community based RA studies tend to consist of smaller proportions of seropositive RA cases at around 50%.

Wolfe 2000 observed that RF concentration in a group of RA cases was strongly related to the number of pack years smoked irrespective of the smoking status of the patient at the time of the study. A longitudinal community study of healthy individuals observed that the persistence of RF significantly increases the risk, 7.5 fold for the development of RA (Halldorsdottir HD, 2000) and there is certainly a strong association between cigarette smoking and RF production in healthy individuals (Tuomi T, 1990). I hypothesise that

prolonged heavy cigarette smoking, but not smoking per se results in increased RF in healthy individuals.

My findings may in part explain the increased mortality observed in RA (Kvalvik AG, 2000), as current data show that continued cigarette smoking throughout adult life doubles age specific mortality rates, nearly trebling them in late middle age (Doll R, 1998). Studies investigating mortality in RA need to consider the smoking history. An example of this is a recent study investigating the mortality of nodular RA patients (Chehata JC, 2001). The commonest cause of death was found to be upper respiratory tract infections. The smoking history in this study was not recorded, however, it is recognised that nodular RA cases have an increased risk of prolonged cigarette smoking than non-nodular RA cases (Harrison, 2001). Therefore the increased mortality observed in nodular RA may in part be secondary to the cigarette smoking history rather than the disease process itself. Another study of Glaswegian RA patients observed a greatly increased mortality rate in patients of the lowest social class compared to those of the highest social class, however cigarette smoking was not considered as a potential confounding factor. This is surprising considering that in the cohort of the poorest RA smokers at least 65% were smoking at the time of RA onset (McEntegart A, 1997).

Recent studies have investigated specific genetic factors underlying a family history of RA. A prospective comprehensive study of familial RA demonstrated no apparent difference in the prevalence of the HLA DR4 shared epitope between familial and sporadic RA (Radstake, 2000) whereas another study observed that the cytokine gene TNF receptor is significantly more prevalent in familial as opposed to sporadic RA (Barton, 2001). It is likely that a familial tendency to RA will consist of a collection of rare haplotypes consisting of a combination of specific HLA groups and cytokine polymorphisms. In light of the findings of a marked difference in pack years smoked between familial and sporadic RA this has

implications for all genetic studies of RA that have not considered the smoking history of the RA patients. To support this statement a recent study (Mattey, 2002) demonstrated that cigarette smoking is a risk for nodular RA and that this risk is independent of RF and a HLA DRB1 (0401)/TNF a6 haplotype.

Further work in chapter 4 considered the influence of a family history of RA and cigarette smoking on the age of onset of RA. This study consisted of RA cases under hospital follow up. The age of onset data was obtained from the case notes and the family history of disease obtained from interviewing the patient. Patients included if they had never smoked or were smoking at the time of their disease onset.

I have demonstrated for the first time that the age of onset of RA differs between sporadic and familial RA cases if they smoke up until the time of disease onset where as lifelong non-smoking sporadic and familial RA cases do not differ in their age of onset. This study highlights the importance of including data on cigarette smoking when considering other risk factors for RA. There appears to be an interaction between a familial tendency towards RA and cigarette smoking as patients with a family history of RA and smoke at RA onset present at the earliest age. This is in keeping with a recent finding of an interaction between cigarette smoking and HLA DRB1 (0401)/ GST M1 Null haplotype in RA (Mattey, 2002). For RA individuals smoking with the HLA DRB1 (0401)/ GST M1 Null haplotype there was a three fold increased risk of developing seropositive RA as opposed to seronegative RA compared to smoking non HLA DRB1 (0401)/ GST M1 Null haplotype RA patients. Whether this can be extrapolated to normal individuals remains to be seen.

The remainder of this thesis investigated potential mechanisms by which cigarette smoking may potentiate the rheumatoid disease process and possible genetic-environmental interactions.

The potential role of NE in the rheumatoid disease process has been extensively reviewed in the introduction. To investigate this process further in RA, serum levels of the IgA-α1PI complex were studied. This study demonstrated that RA patients smokers had significantly higher IgA-α1PI complex levels than non-smoking RA patients. However, I have also found that healthy individuals who smoke have elevated IgA-α1PI complex levels that are comparable with those found in RA smokers. This would suggest that high IgA-α1PI complex levels do not occur as a result of RA per se, but are produced as a result of cigarette smoking. However, I also found that RA disease activity (as measured by ESR levels) is associated with serum IgA-α1PI complex levels independently of current smoking. Non-smoking patients with an ESR >35 had mean IgA-α1PI complex levels equivalent to those of current smokers. These data suggest that high serum IgA-α1PI complex levels can be generated either as a result of current smoking, or by an active disease process in RA patients. I hypothesise that this IgA-α1PI complex formation is induced through oxidative stress that is generated either by smoking, or by an accelerated disease process itself. This process may therefore stop once the patient stops smoking and may represent a reversible process.

I have found that IgA-α1PI complex levels are significantly higher in patients with erosive disease, and suggest that the high complex levels observed in some RA patients who currently smoke may partly explain the association of current smoking with the development of erosive RA. In these patients significant joint destruction could possibly occur through a process involving increased NE. Neutrophil elastase may directly degrade cartilage or indirectly upregulate MMP activity by inhibition of a tissue inhibitor of MMPs (TIMP 1). This study did not directly address whether a reduced capacity to inhibit NE predisposes to rheumatoid joint damage. It is possible that the oxidative process itself rather than the consequences of oxidation to α1PI results in joint damage I confirmed previous findings of

increased IgA-α1PI complex levels in seropositive patients. However I have also found that seropositivity and -α1PI complex levels have *independent* associations with current smoking.

The interaction between GST M1 and cigarette smoking was investigated in chapter 6. This study observed an important environmental-genetic interaction and is the first published data regarding any genetic-environmental interaction in RA. Firstly this study was undertaken in RA females only. This was because smoking and never smoked RA cases were compared as only 10% of male RA cases had never smoked, the numbers of such patients, would have been too small for meaningful statistical analysis. It was demonstrated that women who had smoked compared to those never having smoked had significantly worse rheumatoid disease in terms of Larsen score and HAQ score. This in contrast to the findings of Harrison et al, 2001. Harrison published the only other British study that investigated the role of cigarette smoking in rheumatoid disease severity. This discrepancy could be explained by the fact that the study of Harrison et al investigated the effect of cigarette smoking over a short follow up period of only 3 years. In contrast this study consisted of patients under long-term follow up of a median of 14 years.

It was observed that ever having smoked RA cases had a significantly higher Larsen score than ever smoked RA cases. However, current smokers had a lower Larsen score (although this was not significant) than former smokers despite there being a significant difference in pack years smoked for current smokers and former smokers, 29.3 (14.4) vs. 18.5 (17.5) pack years smoked. The reasons for this are not clear, but one possibility is that former smokers have stopped smoking for a specific reason. Poor health is a powerful trigger for individuals to stop smoking. (Morris JK, 1992). Pulmonary emphysema although caused by smoking is a powerful trigger for smoking cessation (Gorecka D,2001;He Y, 2002). Although 95% of patients with pulmonary emphysema have ever smoked, when the disease develops and requires hospital follow up only 25% of patients continue to smoke (Davis L,

2000). The disease itself clearly modifies the smoking history. There may be common trigger factors for both seropositive RA and emphysema. For example in the population study it was demonstrated that 84% of seropositive RA women are GST M1 null as opposed to 55% of the general population. GST M1 null individuals are at an increased risk of pulmonary emphysema (Harrison DJ, 1997). A recent mortality study of female RA patients has observed that seropositive as opposed to seronegative RA patients have an increased risk of cardiovascular mortality (Goodson, 2002). Interestingly a study of North Americans observed that individuals smoking greater than 20 pack years were at increased risk of heart disease if they were GST M1 null (Li R, 2000). Therefore an important environmental-genetic risk factor for seropositive RA is also evident for both pulmonary emphysema and ischaemic heart disease. The development of either of these diseases is likely to modify the smoking habits of the patients.

It was demonstrated that RA GST M1 null females who smoke are significantly more likely to develop severe erosive RA and have higher RF levels. It is of great interest that GST M1 null females who have never smoked have a very similar disease process to GST M1 +ve females who have never smoked. This suggests that a substrate within tobacco detoxified by GST M1 is responsible for increasing erosive damage in RA rather than a by-product of active RA disease. Currently this substrate is unknown, but would obviously be of great interest in rheumatology research. It is not clear if this substrate influences RA by increased free radical activity subsequent joint damage and consequently increased RF production or that this substrate directly increases RF production which then influences rheumatoid joint disease. The latter is far more likely as RF production is observed far more often in healthy smokers. Secondly in a study of early onset, RA smokers were significantly more likely to be seropositive, but in fact had less severe erosive than seropositive RA cases

who had never smoked (Harrison, 1999). Further studies are required of healthy smokers to determine if GST M1 null individuals have increased RF production.

Independently of RF production it has been demonstrated that GST M1 smokers had more severe rheumatoid joint disease. A caveat here may be that individuals labelled as RF negative may in fact have previously been previously seropositive at the time of developing joint damage and then become seronegative as a consequence of drug therapy or smoking cessation. Large prospective studies will be needed to determine if GST M1 null smoking individuals are at an increased risk of developing more severe rheumatoid joint disease independently of RF production.

One potential mechanism of GST M1 null smokers develop erosive RA independently of RF is increased MMP 1 activity. MMP 1 is an important cause of joint erosions independent of the acute phase response (Cunnane G, 2001). GST M1 inhibits benzo(α)pyrene and other polycyclic hydrocarbons (Rebbeck TR, 1997). Benzo(α)pyrene is an important cause of mutations to P53, an important protein in the pathogenesis of lung and bladder cancer (Sundberg K, 2002;Brockmoller J, 1996). A recent pooled analysis and meta-analysis of GST M1 and bladder cancer observed an additive interaction between GST M1 null and smoking (additive interaction=0.45,95%CI:-0.03,0.93) (Sundberg K, 2002). Recently studies have demonstrated that P53 inhibits MMP 1 activity and that mutagenesis to P53 stops this inhibitory process and subsequently there is an up-regulation of MMP-1 (Sun Y, 1999).

I hypothesised that GST M1 null current smokers are more likely to be susceptible to cigarette induced oxidative stress and therefore have raised serum levels of IgA-α1PI complex. To test this hypothesis I measured the serum levels of IgA-α1PI complex in healthy hospital workers and RA patients and determined the GST M1 genotype in these individuals. I did not demonstrate any relationship between GST M1 null and cigarette

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smoking and raised serum levels of IgA-α1PI complex. This is of potential interest as oxidative stress in the form of hypochlorous acid production is critically important in the development of α1PI oxidation and the formation of the IgA-α1PI complex. One can speculate therefore that GST M1 null smokers are not at increased oxidative stress as compared to GST M1 +ve smokers as a result of increased hypochlorous acid production.

The detoxifying systems that prevent hypochlorous acid accumulation are likely to be important in determining the formation of IgA-α1PI complexes. These systems include both catalase and glutathione peroxidases which break down hydrogen peroxide, reviewed in (Mates M, 2000; Arthur JR, 2000). Interestingly severe RA is associated with low glutathione peroxidase activity, reviewed in (Tarp U, 1994). Conversely superoxide dismutase catalyses the conversion of the superoxide radical to hydrogen peroxide and it has been recently demonstrated that there are important differences in the severity of erosive RA between individuals with differing manganese superoxide dismutase polymorphisms (Mattey DL, 2000).

Glutathione is a ubiquitous antioxidant and has an important role in preventing oxidation of the α1-PI reactive site and this process is thought to be as a result of glutathione scavenging hydrogen peroxide (Gressier B, 1994). The role of glutathione in RA has been reviewed in (Hassan MQ, 2000) and is suggested to be an important defence system in RA. Glutathione is involved in the elimination of reactive electrophiles and in conjugation to lipophilic compounds, to increase their solubility before they are excreted. These detoxyfication reactions are catalysed by glutathione s-transferases, which exist in at least ten isoenzyme forms with different substrate specificities. Therefore any one of a large number of GST genes other than GST M1 may be specifically involved in catalysing the conjugation of glutathione with hydrogen peroxide and therefore preventing oxidative damage to α1-PI as a result of diminished HOCL accumulation. The enzyme methionine-S-oxide reductase has

been observed to restore functional activity to oxidised α1-PI (Morrison HM, 1986) and therefore polymorphisms of the methionine-S-oxide reductase gene may also be important. There are potentially numerous candidate genes that may interact with cigarette smoke to cause α1-PI oxidation and therefore raised serum levels of IgA-α1PI complex levels. I feel these are likely to be important candidate genes for rheumatoid disease progression and the genetic screening of both healthy and RA individuals with raised serum IgA-α1PI complex levels may help to determine genetic factors that determine a poor prognosis in RA.

There are many potential models for erosive disease in RA. Firstly, a model which involves the upregulation of synovial fibroblasts as a consequence of a cytokine dependant response to such potential stimuli as infection or joint damage. These individuals clinically would therefore have obvious synovial proliferation, a raised acute phase response and possibly a genetic trigger factor for a particular cytokine response. Another potential mechanism for erosive joint damage is an upregulation of NE. Therefore this is a neutrophil dependent mechanism. Neutrophils are abundant within the synovial fluid and it has been suggested that they play a role in the development of erosive disease. This particular mechanism may be dependent on RF for continual activation, as RF is responsible for neutrophil degranulation. Cigarette smoking is likely to play an important part in this particular disease pathway. It has been demonstrated in this thesis that RF production in GST M1 null RA patients is significantly more prevalent than in non-smokers and the titres of RF greatly increased. Cigarette smoking can also prime neutrophils increasing degranulation (Gustafsson A, 2000) and inactivate α1PI and therefore greatly increase NE activity. Conversely heavy cigarette smoking is thought to inhibit a cytokine response (Ouyang Y, 2000). Therefore for a number of reasons cigarette smoking may be involved in a NE RA pathway rather than a cytokine dependent pathway (Hutchinson, 2002).

After having completed this thesis I am not convinced that smoking is associated with increased disease severity alone, but rather is related to susceptibility to a particular type of RA. Rather than considering smoking as being superimposed upon a rheumatoid process, making it worse by virtue of the fact that smoking induces RF production I feel it is likely that smoking represents a particular type of RA. This hypothesis is supported by the findings of Saag et al, 1997. Comparing RA patients with the highest quartile of erosive disease smokers were not over represented within this group. Studies such as this are not comparing like with like. Saag studied many persistently seronegative and non-erosive RA patients and the majority of these patients are non-smokers and make up the quartile of patients with the mildest disease. Smokers are under represented within this group and make the majority of the middle two quartiles. If smoking was purely a marker for RA severity one would expect the smokers to be over represented in the highest quartile of RA patients with erosive disease.

It is possible that smoking is associated with a particular type of RA. This type of RA is likely to be more common in men, to be seropositive, nodular, associated with a modest acute phase response and presents at an age that is pack year dependent. It is likely that individuals have always had a tendency of developing a polyarthritis, but the introduction of cigarette smoking bought about a transformation as some of these patients by virtue of their smoking were already seropositive and the potential for disease persistence. Of note is that the classical type of RA has declined over recent years (Silman, 1987) as has persistent smoking.

In summary this thesis has explored the role of cigarette smoking in RA. It has been demonstrated that-

- RA is associated with increased pack years of cigarette smoking.

- Smokers with RA present later than non-smokers.

- RA non-smokers have an increased history of familial disease than RA smokers

- A family history of RA determines earlier age of onset in smokers as opposed to non-smokers.

- Female RA patients who have ever smoked have significantly greater joint damage than female RA non-smokers.

- Erosive RA is associated with raised IgA-α1PI complex levels.

- Raised serum IgA-α1PI complex levels are independently associated with a raised ESR and cigarette smoking.

- Raised serum IgA-α1PI complex levels are not associated with GST M1 null genotype.

- GST M1 null genotype is associated with significantly greater joint damage only in RA smokers.

- GST M1 null genotype is associated with significantly greater RF only in RA smokers.

Finally this thesis has raised the possibility that erosive RA in smokers is the result of at least three different, independent, but potentially interactive mechanisms.

1) GST M1 null associated RF generation.

2) GST M1 null mechanism independent of RF (possibly P53 mutation associated).

3) Generation of raised serum IgA-α1PI complex levels independent of RF (with a subsequent inability to inhibit NE).

Future Work

I hope to extend the work contained within my thesis by further investigating both the roles of α1PI and cigarette smoking in RA. I have commenced some promising work looking at the α1PI promoter polymorphism in RA. Additionally I will be studying genetic factors in RA specifically looking for differences between RA non- smokers and RA patients smoking at the time of diagnosis.

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Smoking and Disease Severity in Rheumatoid Arthritis

Association With Polymorphism at the Glutathione S-Transferase M1 Locus

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Objective. To determine whether the relationship between smoking and disease severity in women with rheumatoid arthritis (RA) is associated with polymorphism at the glutathione S-transferase (GST) M1 locus.

Methods. Genotyping for GSTM1 was carried out using polymerase chain reaction methodology on 164 women with established RA. Smoking history was obtained on each patient. Radiographic damage was measured by the Larsen score, and functional outcome was assessed by the Health Assessment Questionnaire (HAQ). Data were analyzed by multiple regression analyses, with correction for age and disease duration.

Results. Ever having smoked was associated with a worse radiographic and functional outcome than was never having smoked. Both past and current smoking were associated with increased disease severity. Stratification by GSTM1 status revealed that polymorphism at this locus affected the relationship between smoking and disease outcome measures. Patients who lacked the GSTM1 gene and had ever smoked had significantly higher Larsen and HAQ scores than did those who lacked the gene and had never smoked. Radiographic outcome in these patients was worse than that in patients who had the GSTM1 gene and who had

smoked. The associations were not affected by correction for socioeconomic status. Rheumatoid factor (RF) production was found to be associated with smoking in only the GSTM1-null patients.

Conclusion. Our data suggest that disease outcome in female RA patients with a history of smoking is significantly worse than in those who have never smoked. Smoking was associated with the most severe disease in patients who carried the GSTM1-null polymorphism. This association may be due in part to a relationship between the GSTM1 polymorphism and RF production in smokers.

A number of studies have suggested that smoking is a risk factor in susceptibility to rheumatoid arthritis (RA) (1–10). While there have been fewer investigations of the impact of smoking on disease outcome, 3 recent studies have suggested that heavy smoking may influence overall RA severity (11–13). All of these studies reported a relationship between smoking and rheumatoid factor (RF) positivity, nodule formation, and radiographically determined joint damage. Smoking was also associated with increased functional impairment (as determined by scores on the Health Assessment Questionnaire [HAQ]), lower grip strength, and more pulmonary disease (12,13), although these observations may be related to comorbidity effects of smoking rather than the severity of RA per se. In one study, the RF concentration was found to be positively correlated with the number of years smoked (12), while another study showed that the levels of IgA-RF and IgM-RF, but not IgG-RF, were associated with the number of pack-years smoked (13).

The mechanism by which smoking influences RA susceptibility/severity is unclear at present, although it may have direct effects on the disease process by inducing and/or increasing the production of RF or by pro-

ducing alterations in the immune system (3,14–17). The influence of genetic factors on the association between smoking and RA is unknown, although a recent study reported that heavy cigarette smoking was particularly associated with RA in patients who had no family history of the disease (9). We postulate that genes associated with detoxification or activation of chemicals in tobacco smoke will be important. In this regard, our previous finding that increased severity in RA is associated with a null polymorphism at the glutathione S-transferase (GST) M1 locus is of particular interest (18).

The GSTs are a widely expressed group of enzymes that catalyze the detoxification of xenobiotics via glutathione conjugation. They are also believed to play an important role in detoxifying products generated by the activity of reactive oxygen species (ROS). There is evidence that some allelic variants are associated with differences in detoxification efficiency, and various cancer studies have suggested that polymorphism of GSTM1 may influence the ability to detoxify chemicals in cigarette smoke (19–21).

The GSTM1 enzyme detoxifies known or suspected carcinogens found in tobacco smoke. These include benzo[a]pyrene and other polycyclic hydrocarbons (19). The GSTM1 gene has 2 functional alleles (GSTM1*A and GSTM1*B) and a nonfunctional null allele caused by deletion of the GSTM1 sequence. Homozygosity for GSTM1-null occurs in ~45–55% of Caucasians. The GSTM1-null polymorphism has been associated with increased risk of smoking-related cancers (19–25), and a synergistic interaction has been observed between smoking and GSTM1-null on the risk of coronary disease (26).

In this study, we sought to confirm that cigarette smoking was associated with more severe disease in RA and to test the hypothesis that the impact of smoking on disease outcome is associated with polymorphism of the GSTM1 gene which is involved in the detoxification of chemicals in cigarette smoke. The hypothesis was developed a priori with GSTM1-null being considered as the putative high-risk genotype. The study was performed only on female RA patients. Most of the male RA patients (~90%) in the population had been smokers, and it was therefore not possible to test for statistically significant differences between male patients who had smoked and those who had not.

PATIENTS AND METHODS

Patients. The association between GSTM1 genotypes, smoking, and disease severity was studied in 164 unrelated

Table 1. Demographic and clinical characteristics of the 164 female patients with rheumatoid arthritis

	Median (range)	No. (%) of patients
Age, years	57.0 (32–79)	–
Age at disease onset, years	46.0 (19–73)	–
Disease duration, years	11.0 (5–30)	–
Rheumatoid factor positive	–	102 (62.2)
Nodular disease	–	31 (18.9)
Erosive disease	–	148 (90.2)
Previous joint surgery	–	54 (32.9)

female RA patients residing in North Staffordshire, UK. All patients were Northern European Caucasians. The characteristics of these patients are shown in Table 1.

The patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 criteria for RA (27) and were recruited in a clinic that had been established to examine the effects of disease-modifying antirheumatic drugs (methotrexate, sulfasalazine, hydroxychloroquine, gold, and D-penicillamine). About 6.5% of the patients were being treated with corticosteroids. Therapy was administered as clinically indicated.

All patients had been examined annually for at least 5 years, and their disease had been extensively characterized. Outcome measures were recorded at the final review and consisted of assessments of functional status, using the HAQ (28), and radiographic outcome, scoring radiographs of the hands and feet according to the standard radiographs of Larsen et al (29). RF levels were measured by nephelometry; a level >60 IU/ml was considered positive (30).

The Carstairs Deprivation Index (31,32) was used to categorize the socioeconomic status of the patients. Carstairs scores were obtained from the 1991 census small-area statistics for the UK and assigned to each patient based on their enumeration district of residence, which was identified from the postal code address. The index is based on a composite of 4 variables: male unemployment, social class of head of household, overcrowding, and access to a car. The Carstairs scores in the RA population for this study ranged from −4.6 (least deprived) to +5.81 (most deprived).

Smoking history. A current and past smoking history was obtained from each patient. Patients were initially classified by whether they had ever smoked or never smoked. Ever smokers were those who had smoked at least 1 cigarette/day for ≥1 year, but not necessarily during the time of the study. All patients who had ever smoked had started smoking before the onset of RA. Those who had ever smoked were further categorized into past and current smokers. Past smokers were those who had stopped smoking at least 3 months before entry into the study. Of the 35 past smokers, 21 had stopped smoking before the onset of RA. The extent of smoking was quantified in pack-years. One pack-year is equivalent to 20 cigarettes/day for 1 year.

GST typing. Leukocyte DNA was extracted from blood samples that had been collected into tubes containing EDTA. GSTM1 genotypes were defined using a polymerase chain reaction assay that identifies the GSTM1*0, GSTM1*A, and GSTM1*B alleles (33). Patients were classified into those who

Table 2. Frequency of cigarette smoking and seropositivity in 164 female patients with rheumatoid arthritis

Smoking status	No. (%) of patients	% positive for rheumatoid factor
Never	80 (48.8)	51.3
Ever	84 (51.2)	72.6*
Past	35 (21.3)	65.7
Current	49 (29.9)	77.5†

* Odds ratio 2.5 (95% confidence interval 1.2–5.0), $P = 0.006$ versus never-smoked group (adjusted for age and disease duration).
† Odds ratio 3.2 (95% confidence interval 1.3–7.8), $P = 0.004$ versus never-smoked group (adjusted for age and disease duration).

expressed (GSTM1-1) and those who did not express (GSTM1-0) GSTM1.

Statistical analysis. The association of disease severity measures with GSTM1 genotypes and smoking was assessed using multiple regression analyses, with adjustment for the independent variables, age, and disease duration where appropriate. The influence of RF and the Carstairs Deprivation Index were also examined by inclusion as independent variables in some of the regression models. Additional analyses were performed after stratification by GSTM1 status. In some analyses, we also examined the effect of interaction between variables by use of multiple regression models that contained the interaction term as well as the corresponding main effects. Where appropriate, correction for potential multiple testing errors was performed using Holm's procedure (34). All analyses were carried out using the Number Cruncher Statistical Package for Windows (version 6.0.4; NCSS, Kaysville, UT), or the PEPI software package (version 2.0) for epidemiologic analysis (35).

RESULTS

Frequency and extent of smoking in female RA patients. About one-half (51.2%) of the patients had ever smoked (Table 2). The number of smokers had dropped to 29.9% by the time of the study. In this group, the mean duration of smoking and the mean number of pack-years were 38.9 years and 29.3 years, respectively (Table 3), which were significantly greater than in past

Table 3. Extent of cigarette smoking in female patients with rheumatoid arthritis

Smoking status	No. of patients	Years smoked, mean ± SD	Pack-years, mean ± SD
Ever	84	32.5 ± 13.3	24.8 ± 16.1
Past	35	23.5 ± 13.4	18.5 ± 17.5
Current	49	38.9 ± 9.7*	29.3 ± 14.4†

* $P < 0.0001$ versus past-smoker group (corrected for age and disease duration).
† $P < 0.001$ versus past-smoker group (corrected for age and disease duration).

Table 4. Relationship between smoking and radiographic or functional outcome in female patients with rheumatoid arthritis*

Outcome measure	Smoking status			
	Never (n = 80)	Ever (n = 84)	Past (n = 35)	Current (n = 49)
Larsen score	83.1 ± 47.2	104.7 ± 49.9†	112.2 ± 47.7‡	99.3 ± 51.3§
HAQ score	1.39 ± 0.8	1.77 ± 0.8‡	1.86 ± 0.7¶	1.71 ± 0.8#

* P values (versus the never-smoked group) were determined by multiple regression analyses (adjusted for age and disease duration). P_{corr} values were corrected for multiple comparisons by Holm's procedure. HAQ = Health Assessment Questionnaire.
† $P = 0.005$ and $P_{corr} = 0.01$.
‡ $P = 0.002$ and $P_{corr} = 0.006$.
§ $P = 0.05$ and $P_{corr} = 0.05$.
¶ $P = 0.009$ and $P_{corr} = 0.018$.
# $P = 0.02$ and $P_{corr} = 0.02$.

smokers (23.5 years and 18.5 years, respectively). There was a significantly higher frequency of seropositive (RF+) disease among current smokers than among those who had never smoked.

Relationship between smoking and disease severity in female RA patients. The mean Larsen score in patients who had ever smoked was significantly higher than that in patients who had never smoked ($P_{corr} = 0.01$, corrected for age and disease duration) (Table 4). The trend was similar in current smokers ($P_{corr} = 0.05$). The highest mean Larsen score (112.2) was found in past smokers, and this was significantly higher than the Larsen score in patients who had never smoked ($P_{corr} = 0.006$). Using multiple regression analyses (corrected for age and disease duration), we found that in patients who had ever smoked, there was no association between the Larsen score and the number of pack-years smoked ($P = 0.43$).

The HAQ scores showed a trend similar to that of the Larsen scores. Ever, past, or current smokers had significantly increased HAQ scores compared with those who had never smoked (Table 4). Again, no association was found between the HAQ score and the number of pack-years smoked ($P = 0.68$).

Influence of GSTM1 polymorphism on Larsen and HAQ scores. Comparison of Larsen scores in GSTM1-null and GSTM1-1 patients by multiple regression analysis revealed a trend toward more severe disease in GSTM1-null patients, which is similar to that reported previously (18), although this did not achieve significance after correction for age and disease duration (99.4 versus 86.8; $P = 0.1$). There was no significant difference in the HAQ scores between the 2 subgroups (1.57 versus 1.59).

Table 5. Relationship between ever smoking and radiographic or functional outcome in female patients with rheumatoid arthritis, stratified by GSTM1 status*

	Smoking status			
	Never		Ever	
	No. of patients	Mean ± SD	No. of patients	Mean ± SD
Larsen score				
GSTM1-0	43	82.8 ± 49.7	53	112.8 ± 53.3†‡
GSTM1-1	37	83.5 ± 44.9	31	90.8 ± 40.9
HAQ score				
GSTM1-0	41	1.28 ± 0.8	50	1.81 ± 0.8§
GSTM1-1	37	1.50 ± 0.9	31	1.71 ± 0.7

* P values were determined by multiple regression analyses (adjusted for age and disease duration). P_{corr} values were corrected for multiple comparisons by Holm's procedure. GSTM1 = M1 locus of glutathione S-transferase.
† P = 0.006 versus GSTM1-0 + never-smoked group; P_{corr} = 0.012.
‡ P = 0.05 versus GSTM1-1 + ever-smoked group; P_{corr} = 0.05.
§ P = 0.001 versus GSTM1-0 + never-smoked group; P_{corr} = 0.003.

To investigate whether polymorphism of the GSTM1 gene influenced the response of RA to smoking, we stratified patients by GSTM1 status (those expressing versus those not expressing GSTM1) as well as by never smoking or ever smoking. GSTM1-null patients who had ever smoked had significantly higher Larsen (P_{corr} = 0.012) and HAQ (P_{corr} = 0.003) scores than GSTM1-null patients who had never smoked (Table 5). Radiographic outcome in GSTM1-null patients who had ever smoked was also worse than that in GSTM1-expressing patients who had ever smoked (P_{corr} = 0.05), although there was no significant difference in the HAQ scores. In GSTM1-expressing patients, there were no significant differences in the Larsen or HAQ scores between ever smokers and never smokers.

Association between GSTM1 polymorphism, smoking, and RF production. There was a significant association between ever or current smoking and RF positivity (P = 0.006 and 0.004, respectively) after correction for age and disease duration (Table 2). In all smokers, the RF level was also associated with the number of years of smoking (P = 0.02). A linear relationship between RF level and the number of pack-years was not found, but smokers with >20 pack-years had significantly higher RF levels than those with <20 pack-years (264.3 versus 99.9 IU; P < 0.001).

We postulated that the development of more severe disease in GSTM1-null patients who smoked might be associated with a difference in their production of RF. We therefore examined the association between RF status and smoking in patients stratified by GSTM1

genotype. Using logistic regression analysis (adjusted for age and disease duration), we found no significant difference in RF status between ever smoking and never smoking (67.7% versus 51.4%; odds ratio [OR] 1.9, P = 0.2) in GSTM1-1 patients. However, in GSTM1-null patients, there was a difference in RF status between ever and never smoking (75.5% versus 51.2%; OR 3.1, P = 0.01). Division into past and current smokers revealed that this difference was specifically due to a difference between current and never smoking in these patients (83.9% versus 51.2%; OR = 5.1, P = 0.006) (Table 6). No significant difference in RF status was found between current smoking and never smoking in GSTM1-1 patients (64.7% versus 51.4%; OR = 1.7, P = 0.4).

With regard to the amount of RF produced, there was a significant association between current smoking and RF levels in GSTM1-null patients (P = 0.002), but no association in GSTM1-1 patients (P = 0.4). Confirmation of the relationship between current smoking and RF status in GSTM1-null patients (P = 0.005) but not GSTM1-1 patients (P = 0.3) was obtained from a separate cohort of RA patients (n = 134) with early disease (median disease duration 1 year) (data not shown).

Multiple regression models which included RF (positive or negative) as well as GSTM1-null + ever smoking as independent variables showed that RF status had the strongest association with the Larsen score after correction for age and disease duration (Table 7). Nonetheless, after correction for RF status, GSTM1-null + ever smoking remained associated with radiographic outcome, although the significance levels were greatly reduced. A similar observation was found for the HAQ score, although the association with RF status was weaker and age had a more significant effect than disease duration (Table 8). These data suggest that the

Table 6. Relationship between GSTM1 status, current smoking, and RF production in female patients with rheumatoid arthritis*

	RF frequency, no. (%) of patients		RF levels, mean ± SD IU	
	Current smokers	Never smoked	Current smokers	Never smoked
GSTM1-0	26/31 (83.9)†	19/37 (51.2)	284.7 ± 302.9‡	69.8 ± 137.8
GSTM1-1	11/17 (64.7)	21/41 (51.4)	175.4 ± 289.5	74.7 ± 138.1

* Values for rheumatoid factor (RF) frequency are the number positive/number tested (%). P values were adjusted for age and disease duration. GSTM1 = M1 locus of glutathione S-transferase.
† Odds ratio 5.1 (95% confidence interval 1.6–15.3), P = 0.006 versus never-smoked group.
‡ P = 0.002 versus never-smoked group.

Table 7. Multivariate determinants of the Larsen score in female patients with rheumatoid arthritis*

Variable	Regression coefficient	Standard error	P
Constant	9.993	16.387	–
Rheumatoid factor	19.092	6.162	0.002
GSTM1-0 + ever smoked	17.097	7.016	0.033
Disease duration	3.813	0.471	<0.0001
Age	0.379	0.264	0.151

* Multiple regression analysis with the Larsen score as the dependent variable. The P values for rheumatoid factor (RF) and the M1-0 locus of glutathione S-transferase (GSTM1-0) + ever smoked represent the significance of each variable compared with patients negative for that variable or combination of variables (i.e., RF+ versus RF- , GSTM1-0 + ever smoked versus the remainder). The F value for the model was 24.56 ($P < 0.0001$).

association of GSTM1-null + ever smoking with disease severity is not due solely to RF status. However, the possible contribution of other, as yet undiscovered, confounder or modifier variables to the association of this combination variable with the outcome measures cannot be excluded.

Influence of socioeconomic status. We investigated whether socioeconomic deprivation had any effect on disease severity. There was no association between the Carstairs Deprivation Index scores and radiographic outcome in univariate or multivariate models (data not shown). In contrast, the HAQ score was associated ($P = 0.015$) with the Carstairs scores in models corrected for age and disease duration only, but lost significance in multivariate models containing GSTM1-null + ever smoking and RF status (Table 8). Removal of GSTM1-null + ever smoking from the latter model resulted in a weakly significant association between the Carstairs and HAQ scores ($P = 0.04$).

Table 8. Multivariate determinants of the HAQ score in female patients with rheumatoid arthritis*

Variable	Regression coefficient	Standard error	P
Constant	0.198	0.304	–
GSTM1-0 + ever smoked	0.434	0.141	0.002
Carstairs Deprivation Index score	0.040	0.023	0.08
Age	0.019	0.008	0.0002
Rheumatoid factor	0.264	0.121	0.03
Disease duration	0.017	0.009	0.056

* Multiple regression analysis with the Health Assessment Questionnaire (HAQ) score as the dependent variable. The P values for rheumatoid factor and the M1-0 locus of glutathione S-transferase (GSTM1-0) + ever smoked represent the significance of each variable compared with patients negative for that variable or combination of variables. The F value for the model was 9.88 ($P < 0.0001$).

DISCUSSION

We have shown in a well-characterized group of female patients with RA that a history of cigarette smoking is associated with more severe disease, although the mechanism for this effect remains unclear. We also provide evidence that the relationship between smoking and disease severity in these patients is associated with polymorphism at the GSTM1 locus.

Our data suggest that the risk of developing severe disease in female RA patients is increased in those who have the GSTM1-null polymorphism and who have also smoked. The difference between these patients and GSTM1-null patients who had never smoked was highly significant, although significance levels were reduced after correction for RF status. In contrast, there was no significant difference between smokers and nonsmokers who had the functional GSTM1-1 phenotype. Also, in nonsmokers, there was no difference in outcome between GSTM1-null and GSTM1-1 patients. Thus, deletion of the GSTM1 gene per se does not appear to influence RA severity, but does so only in individuals who also smoke. The difference in disease outcome between patients who have never smoked and those who have ever smoked appears to be accentuated in individuals with the GSTM1-null polymorphism. Given the known role of the GSTM1 enzyme in detoxifying chemicals in cigarette smoke, this result suggests that such substrates may have an important influence on the progression of RA.

We confirmed the observations of other studies (11,12), which found that patients who smoked were more likely to be RF positive than were nonsmokers and that the number of years smoked was associated with levels of RF. Of particular interest was the finding that the association between current smoking and RF status was significant only in GSTM1-null patients, where those who smoked had the highest levels of RF. The correlation between smoking and RF has made it difficult to determine the independent predictive value of each of these factors in relation to disease severity. From our data, it is possible to speculate that lack of the GSTM1 enzyme in smokers may promote increased RF production through a failure to detoxify smoke-derived chemicals (or their byproducts), which have the potential to damage IgG. Previous studies have suggested that free radical–mediated alteration of IgG may stimulate the formation of immune complexes with RF antibody, thereby promoting tissue damage during rheumatoid inflammation (36,37).

Saag et al (11) suggested that smoking may be

more important in the initiation of erosive disease than in the perpetuation of the disease process. The lack of an obvious dose-related effect of smoking on the amount of radiographic damage in patients in our study may add some weight to this idea. However, our data also suggest that if smoking is involved in the initiation process, it leads to more severe disease than it does in patients in whom smoking is not involved (particularly if the patients are GSTM1-null).

It might have been expected that increased exposure to cigarette smoke would have led to increased damage and more severe disease. However, the outcome in past smokers was as severe as in those who continued to smoke, even though the number of years smoked and the number of pack-years were significantly lower in the former group. Nonetheless, the mean duration and amount of smoking in the past smokers was relatively high and, compared with other studies, was close to the levels considered to represent a history of heavy smoking (7,11,12). There is evidence to suggest that individuals who smoked in the past continue to produce RF, even after cessation of smoking (12,38). Thus, tissue damage mediated by RF complexes may continue long after the initial stimulus for RF production has been removed.

An alternative explanation for the lack of a dose-related effect could be that some other factor(s) in female smokers predisposes them to the development of more severe RA, especially if they are GSTM1-null. Potential confounders such as body mass index, estrogen levels, oral contraceptive use, or alcohol intake may be important, but without available data on these variables, we were unable to test their effect in our study. However, a recent report from Karlson et al (7) suggested that body mass index and oral contraceptive use were not significant confounders in the association between smoking and RA susceptibility. Other possible confounders such as diet or exposure to environmental toxins may also have some influence but would be difficult to quantify.

The observed associations do not appear to be directly related to social deprivation, since adjustment for socioeconomic status using the Carstairs Deprivation Index did not affect the association between smoking and disease severity in these patients. This is consistent with the report by Saag et al (11), who found that the association of smoking with radiographic changes or RF positivity did not vary with any sociodemographic factors tested. There was no association between the Carstairs score and radiographic outcome with or without inclusion of GSTM1-null + smoking in our regression mod-

els, although we did find a relationship between functional outcome and the Carstairs score after exclusion of GSTM1-null + smoking. This is consistent with previous studies that showed a relationship between functional outcome and social deprivation (39,40). However, those studies did not investigate the influence of smoking, and our data suggest that the relationship between social deprivation and functional outcome is at least partly due to the influence of smoking. It needs to be borne in mind that the HAQ score may not be a specific indicator of the functional impact of RA severity per se. This may be particularly true among patients who smoke, given that smoking is likely to have adverse effects on general health and function.

One limitation of this study is its cross-sectional nature and the difficulty in establishing cause and effect. It has been argued that such studies are subject to misclassification of smoking status and that patients with very mild disease (who no longer seek medical care) or patients with very severe disease (who may have died) are underrepresented (41). While prospective studies may overcome some of these difficulties, they tend to be of relatively short duration and may not properly address the long-term impact of smoking on disease severity. They may also suffer from the difficulty of classifying RA in patients with early inflammatory polyarthritis. We do not believe that misclassification of smoking status is a problem in our current study, especially since we found no difference in disease outcome between past and current smokers. However, our study is clearly limited to women with well-established RA in one particular geographic area, and further studies of different populations are needed to establish the generalizability of these findings.

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(completeness 85%), and they were found to be closely representative of the entire RA registry population. Unlike Mateo and colleagues, we also included patients who had received or were currently receiving medication for osteopenia or osteoporosis.

Despite these differences in patient selection, Drs. Nolla and Mateo, after reanalysis, found the same frequency of reduced BMD as we did, highlighting that bone loss is a frequent problem in male patients with RA. We agree that these cross-sectional observations need further exploration in longitudinal studies, especially to confirm independent associations with corticosteroids and other suggested risk factors for osteoporosis.

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Are cases of rheumatoid arthritis in smokers and lifelong nonsmokers representative of different rheumatoid disease processes? Comment on the article by Harrison et al

To the Editor:

In a recent article in *Arthritis & Rheumatism*, Harrison et al (1) report an association between cigarette smoking and the presence of rheumatoid factor (RF), rheumatoid nodules, and vasculitic complications in patients with polyarthritis. They also observed that, during a relatively short followup time (3 years), joint swelling was significantly reduced in the polyarthritis patients who were smokers. The authors express surprise that, despite a higher rate of seropositivity, the smokers had a lower swollen joint score. This is not, however, an unexpected finding when one considers the pathogenesis of RF in healthy individuals and the consequences of persistent RF in these individuals.

In a study by Heliovaara et al (2), cigarette smoking appeared to be the single most important risk factor for the development of positive RF in healthy individuals. The presence of persistently positive RF in a healthy individual is important because it increases, by 7-fold, the risk for the development of seropositive rheumatoid arthritis (RA) (3). A recent study has demonstrated that heavy cigarette smoking is strongly associated with RA (4), and it was suggested that the mechanism underlying this finding is the association between heavy smoking and RF production.

There are a number of potential mechanisms by which RF may adversely influence the rheumatoid disease process. For example, one of the functions of RF is the induction of neutrophil enzyme release (5). Additionally smokers have primed neutrophils that generate more oxygen free radicals and proteinases (6). Neutrophils are present in large numbers in synovial effusions from patients with erosive RA and contain the proteinases elastase, collagenase, and cathepsin G, which are capable of degrading components of connective tissue matrix (7). Therefore, RA patients who are smokers may have

a predominantly neutrophil-driven erosive disease which is independent of synovial proliferation and joint swelling. This mechanism may also explain Harrison and colleagues' finding of an association between smoking and rheumatoid vasculitis, which is also a neutrophil-driven disease (8).

It is possible that, in smokers who develop an otherwise benign and self-limited arthritis, the disease is transformed by the presence of high-titer RF, which, if not for the smoking history, may never have been present. In contrast, RF may occur as a "reactive" phenomenon in response to synovitis, and therefore in these individuals increased RF production may be a reflection of greatly increased disease severity. These individuals thus could have two mechanisms driving their rheumatoid disease: the process responsible for the synovitis, as well as the resulting RF completing a feedback loop and causing subsequent development of sustained severe disease. Therefore, "pound for pound," an individual with RA who has never smoked and who has strongly positive RF is likely to have more inflammatory disease than an individual with RA who has high-titer RF simply as a result of heavy smoking.

A second potential mechanism by which RA patients who smoke may differ clinically from RA patients who are lifelong nonsmokers is the potential interaction between specific cytokines and cigarette smoke. Relatively few studies have investigated the interaction between cigarette smoke and cytokines, but it has been reported that cigarette smoke contains potent inhibitors of both tumor necrosis factor α (TNFα) and interferon-γ (IFNγ) (9).

Celiac disease is associated with both TNFα and IFNγ (10), and it is therefore of interest that a case–control study demonstrated a strong inverse relationship between current cigarette smoking and celiac disease, with a matched odds ratio of 0.15 (95% confidence interval 0.06–0.38) (11). An association between RA and polymorphism of TNF receptor II in familial as opposed to sporadic RA has been reported (12). Another recent study has demonstrated that heavy cigarette smoking is associated with sporadic as opposed to familial RA (4). It is conceivable that cigarette smoking is inversely related to particular cytokine-driven subtypes of RA, further suggesting that RA patients who are smokers and those who are lifelong nonsmokers are distinct groups.

RF may in fact have 2 roles in RA: first, playing a significant part in disease development and second, triggering a "reactive" and disease-worsening phenomenon in particular individuals who develop a cytokine-driven synovitis. This is not to say, however, that smokers develop less severe erosive disease in the *long term*. Wolfe found that erosive disease in smokers is related to pack-years smoked and that there was a direct relationship between pack-years smoked and RF titer (13). This increase in erosive disease was not associated with a higher acute-phase response, suggesting that erosive and inflammatory processes are discordant in smokers.

Certainly more research on the intriguing relationship between smoking and RA is warranted. It will be interesting to see if in fact RA patients who are lifelong nonsmokers and those who are heavy smokers differ genetically and, perhaps more importantly, in their response to treatment.

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Disease activity and survival in vasculitis: comment on the article by Gayraud et al

To the Editor:

The French Vasculitis Study Group is to be congratulated on the great service it has provided to the medical community with the series of studies of treatment of systemic idiopathic vasculitis. Considerable improvement in both morbidity and mortality has been achieved since the first description of these conditions, which were almost uniformly fatal, although much still remains to be learned about their etiopathologic peculiarities (1). I would point out an error in the article by Gayraud et al (2) in Figure 2B, describing survival in
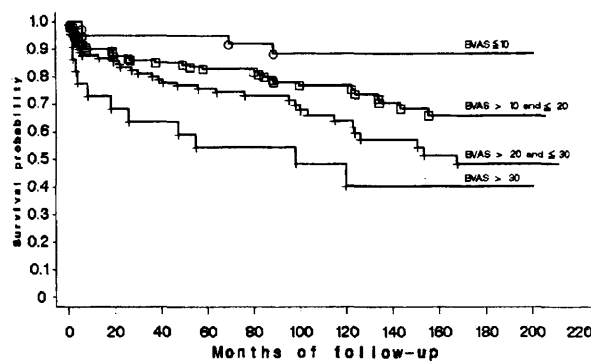

Figure 1. Corrected figure.

vasculitis patients according to their Birmingham Vasculitis Activity Score (BVAS) (3). In the first line, the BVAS should be ≤10 rather than ≥10, as printed.

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Reply

To the Editor:

We thank Dr. Matteson for his kind comments but, most of all, for his eagle eye. Indeed, in Figure 2B, the top curve should read "BVAS ≤ 10." This is corrected in Figure 1 herein.

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Possible association between glucosamine treatment and renal toxicity: comment on the letter by Danao-Camara

To the Editor:

We read with interest the letter to the editor by Danao-Camaro on potential side effects of glucosamine and

MATTERS ARISING

Heavy cigarette smoking and RA

Hutchinson *et al* concluded that prolonged heavy cigarette smoking, but not smoking itself, is strongly associated with rheumatoid arthritis (RA), particularly in patients without a positive family history.[1] The authors proposed that increased rheumatoid factor (RF) production resulting from heavy smoking exposures explains, in part, the relation of increasing cumulative pack years smoked and the greater association with RA.[1]

No data were presented in that study on the extent of smoking and RF positivity or its titres.[1] The proposal[1] would be strengthened if heavy smoking were associated with RF, either when clinical disease began or when patients were studied at hospital rheumatology clinics. Others have proposed that tobacco smoke exposure triggers RF production, thereby contributing to the onset of RA.[2 3] However, no significant association was seen between current smoking and IgM RF positivity in the earlier multicase family study,[2] either among 41 patients with RA or their non-rheumatoid relatives—168 blood and 36 non-blood relatives.[4]

Although heavy cigarette smoking may be associated with RF during clinical disease, it is still relevant to determine whether it is associated with RA, either in the presence or absence of RF positivity. A further question remains as to the sequence of occurrences. Does heavy smoking first induce RF production, which later contributes to RA?[1 3] Alternatively, might RA be induced first and RF produced later? Prospective, rather than cross sectional, studies are needed to answer these questions. Prospective data suggest that reported smoking of 30 or more cigarettes daily (CS 30+/day) predisposes to RA risk independently from RF positivity or positive family history.[5 6]

These complex relationships were investigated in a case-control study nested within a community based cohort (n=21 061 adults)

enrolled in 1974. For each of the 18 male and 36 female unrelated incident patients who satisfied American College of Rheumatology criteria for RA, identified in 1994, four controls from the entry cohort were matched for age, sex, and race (all white subjects).[5 6] Table 1 shows the number of patients before they developed RA and their respective controls who reported heavy cigarette smoking (CS 30+/day) at baseline. Heavy smoking was not associated with pre-RA RF+ status, but was associated significantly (p=0.001) with patients who were RF– at baseline. The highest observed odds ratio (OR) was in 15 sets in which the patient was RF– at baseline and continued to be RF– after active disease developed (OR 21.5, 95% CI 1.9 to 122.5, p=0.005). The ORs were similar for sets in which the patients had positive or negative FDR status, but was significant (p=0.012) only in the larger FDR– subset (table 1).

The hypothesis that cigarette smoking contributes to RA partly by RF production[1 3] is attractive. However, critical substantiation in prospective and cross sectional studies is currently lacking. Available prospective data (table 1)[5 6] suggest that alternative mechanisms may be more likely. For example, long term cigarette smoking causes general vascular endothelial damage,[7] and smoking is significantly associated with vasculitis in active RA.[8 9] Heavy smoking was proposed[4] to contribute to RA risk through its endothelial and microvascular effects, perhaps through nitric oxide pathways,[10] rather than by RF production primarily.[1 3]

Whether or not heavy smoking differentially associates with RA depending upon family history of disease[1] is as complex as the dilemmas of RF contributions to onset (table 1). Our FDR+ female patients had a significantly (p<0.001) younger mean age at clinical onset (45.6 years) than their counterparts (57.1 years). Might such earlier onset of RA among patients with a positive family history, as also noted by Hutchinson *et al*,[1] have influenced their behaviour to lower cumulative exposures to cigarette smoking compared with their counterparts?[1]

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Authors' reply

We read the letter of Masi *et al* with interest and are pleased to have an opportunity to discuss the questions they have raised. Our study group was derived from an area of northwest England made up principally of people in a lower socioeconomic class, in contrast with other UK studies.[1] Although we did not record the presence of rheumatoid factor (RF) in our patients for the purpose of this study, seropositivity in our RA patient group was high, approximately 80–90%. This is comparable with Glasgow, an area in Scotland with a similarly high level of social deprivation, where 96% of randomly selected patients with RA were found to be seropositive.[2] We therefore decided to compare the smoking history of familial and sporadic patients with RA rather than compare seropositive and seronegative patients.

Published reports almost uniformly suggest that cigarette smoking is associated with seropositive rather than seronegative RA. Cigarette smoking is associated with the development of seropositivity in healthy subjects[3 4] and, furthermore, there is a dose related phenomenon for the development of seropositive RA.[5] It has also been established that the development of seropositive RA is greatly increased in healthy subjects who are persistently seropositive.[6] Wolfe noted a significant trend in patients with RA of

Table 1 Numbers of pre-RA cases and matched controls reporting heavy cigarette smoking (CS 30+/day) at baseline by relevant categories and odds ratios (ORs) with 95% confidence intervals (95% CIs) for developing ACR+ rheumatoid arthritis

Categories	Pre-RA cases		Respective matched controls			
	Number	CS 30+/day (%)	Number	CS 30+/day (%)	OR	95% CI
Total subjects	54	13 (24)	216	19 (9)	3.3	1.4 to 7.7
Men	18	8 (44)	72	14 (19)	3.3	1.0 to 11.4
Women	36	5 (14)	144	5 (3)	4.5	1.0 to 19.4
FDR+*	11	5 (45)	44	9 (20)	3.2	0.7 to 16.3
FDR–	43	8 (19)	172	10 (6)	3.7	1.2 to 11.2
Pre-RA RF+	12	2 (17)§	48	7 (15)§	1.2	0.2 to 7.9
Pre-RA RF–	42	11 (26)	168	12 (7)	4.6	1.5 to 10.2
Entry and post-RA RF–	15	4 (27)	60	1 (2)	21.5	1.9 to 122.5
Conversion of pre-RA RF– to RF+†	27	7 (26)	108	7 (6)	5.1	1.4 to 18.5
FDR– and pre-RA RF–	33	7 (21)	132	5 (4)	6.8	1.8 to 27.4
Mild course of RA‡	19	8 (42)	76	11 (14)	4.3	1.2 to 15.1
Non-mild course of RA‡	35	5 (14)	140	8 (6)	2.8	0.7 to 10.2

*FDR+ is a positive history of RA in a first degree relative as determined for patients in 1997, and reported in 6/18 (33%) male patients and 5/36 (14%) female patients.
†Conversion of RF– at baseline to RF+ after clinical onset of RA.
‡Course of RA over 3–20 (median 11) years of clinical disease was determined by the patients' rheumatologist according to predefined criteria.
§No association of CS 30+/day with pre-RA RF+ (p=0.99).

increasing RF titre with pack years smoked.[7] Yet although the development of rheumatoid joint erosions, nodules, and disability was significantly increased by cigarette smoking, he found that this was independent of RF production.

We suspect that cigarette smoking and RF are strongly interlinked, but other mechanisms, as suggested by Masi, may also be at work. For example, cigarette smoke contains numerous oxidising agents that can inactivate α_1-proteinase inhibitor (α_1-PI),[8] the natural inhibitor of neutrophil elastase (NE), a serine proteinase that can degrade articular cartilage.[9] Cigarette smoke can also prime neutrophils to degranulate and discharge NE,[10] activate macrophages to produce matrix metalloproteinases,[11] up regulate production of interleukin 1β and interleukin 8[12] and down regulate interleukin 1 receptor antagonist,[13] and interleukin 10.[14] Furthermore, cigarette smoking induces disease processes in a specific dose dependent fashion (independent of current smoking status), such as pulmonary emphysema, in which there is increased neutrophil priming, increased oxidised α_1-PI and α_1-PI-NE complexes (indicative of increased NE activity).[8] Therefore a heavy smoker may have an otherwise benign short lived inflammatory arthritis modified by the mechanisms outlined above and develop RA.

Whether RA increases or decreases cigarette consumption remains uncertain. Our controls had a pack year total estimated at entry to the study and not at the time of their disease onset. We are, however, unaware of any data to suggest that RA increases cigarette consumption. Indeed, a study by Harrison et al observed that 18% of all smokers with polyarthritis stopped smoking within three years of disease onset as opposed to <1% of non-smoking patients who started smoking during this period.[15]

Other important questions remain unanswered. For example, does increased cumulative cigarette consumption increase RA susceptibility independently of RF production? (Data presented here by Masi et al only consider cigarette consumption at one time point.) If so, do these subjects have an increased prevalence of circulating levels of α_1-PI-NE complexes (levels of oxidised and inactivated α_1-PI complexes, and therefore pulmonary emphysema?

We welcome the heightened interest in the relationship between smoking and RA and look forward to the establishment of new studies designed to answer some of the interesting questions raised by recent studies.

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Rheumatoid arthritis associated with ulcerative colitis

I was interested to read the letter on "Rheumatoid arthritis associated with ulcerative colitis" by Boyer et al published recently in the Annals,[1] and would like to make the following comments. Studies in patients with established Crohn's disease (CD) have generally supported the predominance of Th1 responses.[2] [3] In ulcerative colitis, although enhanced humoral immunity has been described, evidence for classical Th2 predominance remains to be demonstrated. On the other hand, it has been shown that interleukin 15 is overexpressed in the inflamed mucosa of patients with inflammatory bowel disease at the level of macrophages.[4] Similar findings have been reported in patients with rheumatoid arthritis (RA).[5]

As shown in this case, it is sometimes quite difficult to distinguish by clinical manifestations alone between two diseases which start almost at the same time. However, the presence of a positive rheumatoid factor and DR1 genotype are arguments for RA. The existence of polymorphisms affecting other genes may take place in such type of arthritis.[6]

Results obtained with anti-tumour necrosis factor monoclonal antibody to prevent mucosal inflammation in CD,[7] suggest that such an approach may also be of interest in this unusual situation.

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Authors' reply

We thank Dr Mosquera-Martinez for his letter and are happy that our report has stimulated active discussion and suggestions.[1] Indeed, control of disease was difficult even when combining methotrexate 15 mg/week IM, salazopyrine 3 g/day, and prednisone 10 mg/day. The patient still had active arthritis affecting wrists and hands with an erythrocyte sedimentation rate (ESR) of 47 mm/1st h. Furthermore, she also had active colitis, and current treatment prevented surgery for colon anastomosis.

Accordingly, infliximab was started following the now classical rheumatoid arthritis protocol.[2] Seven months later, steroids could be stopped. Surgery for permanent colon anastomosis could then be performed with success and with no healing delays. When last seen in July 2001, she showed major improvement, with no pain at night and no morning stiffness. She had gained weight and had no sign of active colitis. The ESR was 26 mm/1st h and C reactive protein <4 mg/l.

Such follow up extends the concept of common mechanisms between rheumatoid arthritis and ulcerative colitis. Both diseases appear to depend, at least in part, on the contribution of tumour necrosis factor α.

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Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA

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Abstract

Objectives—To investigate the potential relation between cumulative exposure to cigarette smoking in patients with or without rheumatoid arthritis (RA) and a positive family history of the disease.

Methods—239 outpatient based patients with RA were compared with 239 controls matched for age, sex, and social class. A detailed smoking history was recorded and expressed as pack years smoked. Conditional logistic regression was used to calculate the association between RA and pack years smoked. The patients with RA were also interviewed about a family history of disease and recorded as positive if a first or second degree relative had RA. The smoking history at the time of the study of the patients with RA with or without a family history of the disease was compared directly with that of their respective controls. Patients with RA with or without a family history of the disease were also compared retrospectively for current smoking at the time of disease onset.

Results—An increasing association between increased pack years smoked and RA was found. There was a striking association between heavy cigarette smoking and RA. A history for 41–50 pack years smoked was associated with RA (odds ratio (OR) 13.54, 95% confidence interval (95% CI) 2.89 to 63.38; p<0.001). The association between ever having smoked and RA was modest (OR 1.81, CI 1.22 to 2.19; p=0.002). Furthermore, cigarette smoking in the patients with RA without a positive family history of RA was more prevalent than in the patients with a positive family history of RA for ever having smoked (72% *v* 54%; p=0.006), the number of pack years smoked (median 25.0 *v* 4.0; p<0.001), and for smoking at the time of disease onset (58% *v* 39%; p=0.003).

Conclusions—Heavy cigarette smoking, but not smoking itself, is strongly associated with RA requiring hospital follow up and is markedly more prevalent in patients with RA without a family history of RA.

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Although both genetic and environmental factors are thought to contribute to the development of rheumatoid arthritis (RA),[1] the cause of this disease remains unclear. The genetics of RA are complex.[2] At the simplest level, there is an increased risk of hospital based RA if a family member also has the disease (odds ratio (OR) 6).[3] The natural history of RA, however, suggests that the disease may be initiated by relatively contemporary environmental factors. Rheumatoid arthritis was first described as recently as 1800 and examination of the visual arts before this time has failed to show any evidence of RA.[4] The low concordance for RA in monozygotic twins[5] highlights the importance played by environmental factors.

One potential environmental agent is cigarette smoking. A Norwegian study reported that men who were current smokers had an increased risk of developing seropositive RA (OR 4.77, 95% CI 2.09 to 10.9).[6] However, this study, did not quantify the number of cigarettes smoked and therefore could not address the potential effects of cumulative exposure to cigarette smoke. In other smoking related diseases, such as emphysema,[7] lung cancer,[8] and ischaemic heart disease,[9] the risk of disease with increasing cumulative exposure to cigarette smoke is increased. In a North American study of female health workers an increased risk of 49% for developing seropositive RA was found in those smoking 25 cigarettes a day for more than 20 years.[10]

Both the above studies investigated community based RA, where there is a lower prevalence of disability than in hospital based patients with RA.[11] This is relevant as current cigarette smokers of 25 pack years or more are 2.4 times more likely to have erosive rather than the milder non-erosive RA than subjects who have never smoked.[12] We therefore undertook a study to test the hypothesis that a high cumulative exposure to cigarettes is associated with RA requiring hospital outpatient treatment, and to examine the potential role of a positive family history of RA underlying this.

Methods

Two hundred and thirty nine unrelated patients with RA (age range 28–87) attending rheumatology clinics in two Merseyside hospitals and fulfilling the 1987 American Rheumatism Association criteria for RA[13] were studied. The patients' age and age of disease onset were recorded. Social class was defined by the Office of National Statistics Classification of Occupations[14] based on the highest employment history of the subject before the age of onset of

Table 1 Characteristics of the patients with rheumatoid arthritis and controls studied

	Patients	Controls
Number	239	239
Sex (No (%))		
Male	79 (33)	79 (33)
Female	160 (67)	160 (67)
Age (years)		
Mean (SD)	60.5 (11.8)	60.5 (11.8)
Age at onset (years)		
Mean (SD)	46.9 (13.2)	NA
Disease duration (years)		
Mean (SD)	13.6 (9.9)	NA
Social class (No (%))		
Classes I–II	29 (12)	29 (12)
Classes IIIN–IIIM	86 (36)	86 (36)
Classes IV–V	124 (52)	124 (52)

disease or in the case of a woman who had never worked, based on her partner's occupation. This classification allocates subjects in one of six social classes according to occupation as follows: I = professional, II = managerial, IIIN = non-manual skilled, or IIIM = manual skilled, IV = partly skilled, and V = unskilled. A positive family history of RA was defined according to strict criteria: (*a*) a first or second degree relative with a history of RA; (*b*) this relative must have been receiving regular follow up in a rheumatology department, received a disease modifying antirheumatic drug, or have the deformities or nodules typical of RA; (*c*) as the population in this region is relatively static, case notes of the relative were checked to verify the history of RA, where possible.

A comprehensive past and current smoking history was taken from the time of inclusion to the study. Cigarette consumption was quantified in pack years. One pack year is equivalent to 20 cigarettes smoked a day for one year. A current smoker was taken to be a smoker of at least one cigarette a day for one year. An ex-smoker was defined as a person who had stopped smoking for at least three months. Control subjects were seen in a north Staffordshire dermatology outpatients clinic. Specifically, the controls all had benign non-inflammatory skin lesions. They were investigated in an identical manner to the

patients with RA (by JTL). The patients with RA and controls were then matched for age, sex, and social class.

Conditional logistic regression was used to calculate the odds ratio for having RA. Between patients with RA and matched controls, frequencies were compared using the sign test for categorical data and the Wilcoxon signed rank test for continuous data. Between groups within patients with RA, frequencies were compared with the χ^2 test and continuous data using the two sample t test, where a Gaussian distribution could be assumed, or the Wilcoxon two sample test otherwise (indicated by the quoting of medians rather than means). Statistical significance was set at the 5% level.

Results
Two hundred and thirty nine patients with RA, with a mean (SD) age of 60.5 (11.8) years were studied. Table 1 summarises their demographic details.

CASE-CONTROL STUDY: SMOKING STATUS AT TIME OF STUDY
Patients with RA were significantly more likely to be current smokers than controls matched for age, sex, and social class (table 2). Of the patients with RA, 100 (42%) were current smokers *v* 52 (22%) of the controls (p<0.001). There was no significant difference in the proportion of subjects who were ex-smokers: 55 (23%) of the patients with RA *v* 72 (30%) of the controls (p=0.09). Of the patients with RA, significantly fewer had never smoked: 84 (35%) *v* 115 (48%) of the controls (p=0.005). The difference between pack years smoked in the patients with RA and controls was highly significant (a median value of 18.0 *v* 1.0; p<0.001.).

There was a dose dependent association between pack years smoked and RA (summarised in table 3). The association between RA and ever having smoked and RA was modest (matched OR 1.81, 95% CI 1.22 to 2.19; p=0.002), but there was a strong association between heavy cigarette smoking and RA with 41–50 pack years (matched OR 13.54, 95% CI 2.89 to 63.38; p<0.001).

Table 2 Comparison of smoking history between patients with rheumatoid arthritis and dermatology controls

	Current smokers (No (%))	Ex-smokers (No (%))	Never smoked (No (%))	Pack years (median (semi-IQR)) *
Patients (n=239)	100 (42)	55 (23)	84 (35)	18.0 (18.5)
Controls (n=239)	52 (22)	72 (30)	115 (48)	1.0 (8.5)
Significance (p value)	<0.001²	0.09†	0.005†	<0.001‡

*Semi-IQR = semi-interquartile range.
†Resulting from a sign test.
‡Resulting from a Wilcoxon signed rank test.

Table 3 Odds ratio for having rheumatoid arthritis with pack years of cigarettes smoked

Pack years	Patients (%)	Controls (%)	Odds ratio* (95% CI)†	Significance (p value)
1–10	112 (47)	161 (67)	0.80 (0.44 to 1.50)	0.496
11–20	15 (6)	35 (15)	0.55 (0.26 to 1.16)	0.111
21–30	34 (14)	26 (11)	1.76 (0.95 to 3.29)	0.068
31–40	30 (13)	7 (3)	5.72 (2.28 to 14.36)	<0.001
41–50	27 (11)	4 (2)	13.54 (2.89 to 63.38)	<0.001
>50	21 (9)	6 (3)	8.41 (2.45 to 28.84)	<0.001
Ever smoked	155 (65)	124 (52)	1.81 (1.22 to 2.19)	0.002

*Calculated using conditional logistic regression.
†95% CI = 95% confidence interval.

COMPARISON OF SMOKING BETWEEN PATIENTS WITH RA WITH OR WITHOUT A FAMILY HISTORY OF RA
One hundred and forty three (60%) patients with RA had no family history of RA (FH −ve RA). When these patients were compared with the patients with RA with a positive family history of RA (FH +ve RA) there were no apparent differences in age, sex or social class (table 4). A comparison of the smoking history was made between these two groups. The RA FH −ve patients smoked significantly more than RA FH +ve patients. More RA FH −ve patients had ever smoked (72% *v* 54%; p=0.006), were smoking at the time of disease onset (58% *v*

Table 4 Comparison of demographic and smoking history between patients with rheumatoid arthritis with or without a family history of the disease

	Family history +ve (n=96)	Family history −ve (n=143)	Significance (p value)
Sex (No (%))			
Male	23 (24)	29 (20)	
Female	73 (76)	114 (80)	0.499*
Age at onset (years)			
Mean (SD)	44 (12.5)	49 (13.5)	0.003†
Smoking at diagnosis (No (%))			
Yes	37 (39)	83 (58)	
No	59 (61)	60 (42)	0.003*
Social class (No (%))			
Classes I–II	11 (11)	19 (13)	
Classes IIIN–IIIM	37 (39)	47 (33)	
Classes IV–V	48 (50)	77 (54)	0.656*
Number of pack years			
Median (semi-IQR)¶	4.0 (13.5)	25.0 (21.5)	<0.001‡

*Resulting from a χ^2 test.
†Resulting from a two sample *t* test.
‡Resulting from a two sample Wilcoxon test.
¶Semi-IQR = semi-interquartile range.

39%; p=0.003), and had smoked a greater number of pack years (median of 25.0 *v* 4.0, p<0.001). The median RA FH −ve patients' cumulative exposure to cigarettes was 25.0 pack years more than the controls with dermatology disease (25.0 RA *v* 0.0 controls; p<0.001), whereas there was no difference for RA FH +ve cases (4.0 pack years *v* 5.0 controls; p=0.980). Furthermore, the mean age at onset of RA FH +ve cases was significantly lower (44 (12.5) years, median age 43, range 18–73) than the FH −ve group (mean age 49 (13.5) years, median age 50, range 19–78; p=0.007) and disease duration was longer in RA FH +ve patients (mean 17.6 years (9.5)) than in RA FH −ve cases (mean 10.8 (9.9) years; p<0.0001).

COMPARISON OF PATIENTS WITH RA NEVER HAVING SMOKED AND PATIENTS WITH RA SMOKING AT THE TIME OF DISEASE ONSET

One hundred and twenty (50%) patients with RA were currently smoking at the time of disease onset. Eighty four (35%) of the patients with RA had never smoked. The mean age of onset differed significantly between these two groups: 43.2 years (13.8) in the patients with RA never having smoked (median age 42, range 18–70) in comparison with a mean age of 47.6 (12.3) (median age 47, range 18–72) in the patients with RA smoking at the time of disease onset (p=0.001). Significantly more of the patients with RA who had never smoked (46 (54%)) had a positive family history of RA than the patients with RA smoking at disease onset (39 (33%); p=0.004).

Discussion

In this study we examined the relation between cigarette smoking in patients with RA attending rheumatology outpatient clinics in Merseyside, an industrial region of the north of England. We found a dose dependent association between pack years smoked and RA. A high cumulative exposure to cigarette smoke was strongly associated with RA. Additionally, the patients with RA with a family history of RA were significantly less likely to be current cigarette smokers at the time of disease onset

and smoked significantly fewer pack years than the RA group without a family history of RA.

The inclusion of controls from a different region of the north of England is unlikely to have introduced a bias as the control subjects were matched for age, sex, and social class, which are the principal determinants of the prevalence of cigarette smoking.[15] It may be that dermatology patients smoke in a different fashion than the general population, but we feel that this is unlikely as we excluded patients with inflammatory skin lesions such as psoriasis, a condition associated with excess tobacco consumption,[16] and atopic eczema, which is associated with asthma and therefore disassociated from heavy cigarette smoking.

Control subjects with malignant skin lesions were also excluded, as such lesions are significantly more common in outside manual workers, such as agricultural workers[17] and welders.[18] Also, the smoking history of people with these particular occupations may not be similar to those of manual indoor workers, who may have smoking constraints imposed upon them. Furthermore, the control subjects matched the RA FH +ve group for pack years smoked. This is important as the smoking history of the RA FH +ve group is typical of that of the adult population aged 18–60 years for our region of the north west of England, with 39% of the RA FH +ve group smoking at the time of diagnosis compared with 39% of adults (18–60) who are current smokers in our region of Merseyside (St Helens).[15]

We confirmed previous findings that cigarette smoking itself is modestly associated with RA. For ever having smoked, the association with RA is modest (matched OR 1.81, 95% CI 1.22 to 2.19). This is a similar association to that observed by Symmons *et al* in a community based study[19] (OR 1.66, 95% CI 0.95 to 3.06). However, the number of cigarettes smoked was not quantified in Symmons's study, or in other studies[6 20] investigating the association between cigarette smoking and RA. Therefore no direct comparison can be made between these studies and our own observations about the risk for heavy cigarette smoking. Our study shows the need to quantify the number of cigarettes smoked when studying the relation between cigarette smoking and its association with RA. The strong association with a high cumulative exposure to cigarettes may partly underlie the observation that the incidence of RA increases with age. In keeping with this hypothesis, a Finnish study observed a 20-fold increased incidence of RA in men currently smoking as opposed to men who had never smoked after individual follow up of 14 or more years.[20]

We found a modest non-significant association between smoking and RA in subjects who had smoked less than 30 pack years. However, we found that prolonged exposure to cigarette smoke resulted in a strikingly increased risk of developing RA for patients with a 41–50 pack year cigarette habit. This has implications for studies in countries in which heavy cigarette smoking is extremely uncommon. An example of this is a large prospective study over 20 years

in Finland designed to examine the relation between cigarette smoking and the development of RA.[20] The study found no relation between cigarette smoking and RA in Finnish women. However, only 2.5% of the women studied were current cigarette smokers of more than 15 cigarettes a day and few would be exposed to more than 30 pack years of cigarettes. In contrast, a recent large North American community based study did observe a significantly increased risk of RA in women smoking more than 25 cigarettes a day for more than 20 years.[10] The increased risk observed in the North American study is modest, but this study had a low response rate (22%). Nonresponders in RA studies tend to be older,[6] have greater comorbidity, and are poorer than responders.[21] It is likely that smokers are over-represented in those particular non-responder groups and therefore the association with RA might have been underestimated. It is noteworthy that our findings apply to the patients with more severe RA seen in patients attending out-patients clinics, and are not necessarily true for milder, community based RA. Most studies investigating the association between cigarette smoking and RA have been community based studies.[6 10 19 20 22 23]

Genetic factors are of clear importance in the development of RA. Family history of RA was chosen as a simple surrogate marker for genetic predisposition. The criteria for "RA" in relatives were made as stringent as possible, to avoid the common mistake of calling a degenerative disease "rheumatism" or "RA". Forty per cent of our RA group had a family history of RA. This is higher than previously reported in the literature. However, these studies included first degree relatives only.[5]

We found that RA FH +ve patients had an almost identical pack year history to that of their respective controls and smoked significantly less than the RA FH –ve patients. On average, the patients who were RA FH +ve had had the disease for longer (6.8 years) than patients who were RA FH –ve. In keeping with this finding, patients with RA with disease associated major histocompatibility complex genes appear to develop RA before those who do not.[24] These data also suggest that RA does not predispose patients to smoke more heavily, as has been previously suggested,[25] because the FH +ve group had had RA the longest but smoked the least. Our observation that patients with RA who have never smoked have a significantly increased prevalence of a family history of RA than those who smoke at the time of diagnosis would affect the interpretation of the many studies that have investigated the genetics of RA without recording the smoking history.

The reasons why prolonged cigarette smoking should be strongly associated with the development of RA are not clear. A recent study by Wolfe found that rheumatoid factor concentration in a group of patients with RA was related to the number of pack years smoked, irrespective of the smoking status of the patient at the time of the study.[26] A longitudinal community study of healthy subjects found that the persistence of rheumatoid factor significantly increases the risk, 7.5-fold for the development of RA,[27] and there is certainly a strong association between cigarette smoking and rheumatoid factor production in healthy subjects.[28] We propose the hypothesis that prolonged heavy cigarette smoking, but not smoking itself, results in increased rheumatoid factor production, and that in part this explains the relation of increasing pack years smoked and the association with RA. Surprisingly, no studies of other possible mechanisms to explain the association between cigarette smoking and RA have been carried out.

This study may, in part, explain the increased mortality found in RA,[29] as current data show that continued cigarette smoking throughout adult life doubles age-specific mortality rates, nearly trebling them in late middle age.[30]

This study was not designed to consider whether an association between cigarette smoking and RA is causal. As possible evidence, we would cite the reduced incidence of RA in the UK over recent years,[31] coinciding with an overall reduction in smoking evident over the past 30 years. In 1971 45% of the adult population were current smokers,[32] as compared with 28% in 1996.[15] An unfortunate corollary is that this trend may be reversed in the years to come as the proportion of young girls smoking is increasing.[15] If the recent trend for increased cigarette consumption in young woman continues, a new epidemic of RA may occur, not immediately, but in the time it takes to smoke the equivalent of 30 or more pack years of cigarettes.

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large, that this is part of the problem, this practice is unlikely to change.

By setting forth this model we can now investigate it. We are making efforts to do this, and we hope that quality researchers such as Drs Barnsley and Bogduk will engage in such efforts as well.

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Authors' reply to Dr Radanov

Dr Radanov's expressed concerns and cry for *auto-da-fé** are based on his perception that our biopsychosocial model is one of malingering as an explanation for the late whiplash syndrome. As we have explicitly stated, in both our current article and in a previous review on this topic, we reject a model based on malingering and we consider this to be a rare or uncommon presentation.[1] Dr Radanov's concerns are therefore misplaced. That Dr Radanov is unable to appreciate how our biopsychosocial model presents alternatives to the otherwise unhelpful, unidimensional, and dichotomous approaches taken by himself and others is a problem for him, but one which we cannot ameliorate in the space available. We thus refer him to a more comprehensive resource.[2]

Once again, we reject the view that the chronic pain of whiplash is due to an enigmatic and inexplicable chronic injury, and we simultaneously reject the view that the best explanation (or even a common explanation) for the late whiplash syndrome is malingering or psychological models that place the pain "all in one's head". The biopsychosocial model includes physical sources for pain, and incorporates psychosocial factors to explain both the severity and attribution of the pain, as well as further behaviour enacted upon this substrate of otherwise benign physical sources of pain. Thus we maintain that the most helpful focus of discussion and research should be on identifying how the various elements of the biopsychosocial model explain

the variance in epidemiology of the late whiplash syndrome, and why, even within a given culture some accident victims recover quickly and others do not. Dr Radanov's views may be coloured by the relatively benign nature of the problem he sees in Switzerland. Even with an advertising campaign to recruit subjects, the Swiss outcomes were very much better than those currently being described in North America. We maintain that the Swiss effort at understanding these issues has been a start, but is a mere footstep in a much longer journey of inquisition.

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Rheumatoid arthritis, poverty and smoking

Maiden *et al* raise a number of important and interesting points in their paper "Does social disadvantage contribute to the excess mortality in rheumatoid arthritis patients?"[1]

They have observed that mortality in rheumatoid arthritis (RA) correlated with social grouping on the west coast of Scotland. Patients with RA of the lowest socioeconomic classes have an increased mortality when compared with patients of a higher socioeconomic class. Moreover, RA was more prevalent in patients with RA of lower socioeconomic class. We propose that these two important observations can both be explained by cigarette smoking.

The authors commented that cigarette smoking was more prevalent in the patients with RA of lower socioeconomic class in their study. In Britain there is a marked difference in smoking prevalence between social classes. In the 1996 census 41% of lower social class men (social class 4) were current smokers, with only 12% of men in the highest social class (social class 1) currently smoking.[2] Cigarette smoking kills 120 000 people a year in Britain.[3] Most of these deaths are as a result of cardiovascular disease, respiratory disease, and lung cancer. Maiden *et al*[1] observed that 65% of the deaths in their study occurred as a result of these diseases. Current data show that continued cigarette smoking throughout adult life doubles age-specific mortality rates, nearly trebling them in late middle age.[4] Cigarette smoking is associated with an increased risk of RA in both men[5] and

women[6]. The increased mortality seen in patients with RA of low socioeconomic status could be explained in part by cigarette smoking, and that cigarette smoking itself might have contributed to the excess RA seen in the most socially deprived.

Since the poorest in our society appear to have an increased risk of RA, studies designed to identify risk factors for RA may best be focused on those with the highest risk. Cigarette smoking may be especially important to study, because its most powerful effect may be seen in the poorest socioeconomic population with RA. Laudable attempts to study the epidemiology of RA in Britain have been set up. One example is the Norfolk Arthritis Register. However, we would suggest such populations, in which there are a large proportion of higher socioeconomic groups, are unrepresentative of the large industrial cities in Britain. In 239 patients with RA in the Merseyside region under hospital follow up, the social class of our patients was identified using the Office of National Statistics classification of occupations.[7] The patients with RA in Merseyside were of significantly lower social class than the patients with inflammatory polyarthritis studied in Norfolk.[8] Table 1 summarises these findings. If the findings reported by Maiden *et al*[1] are supported by further studies, there would seem to be significant differences in incidence, severity, and mortality in RA according to socioeconomic profiles. This would mean that increased resources should be allocated to regions of greatest need and not, as at present, to areas where socioeconomic class is highest, such as the south of England.

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Table 1

	Social class† 1–2 No (%)	Social class 3N–3M‡ No (%)	Social class 4–5 No (%)
Inflammatory polyarthritis cases Norfolk and Norwich[8] (154)	51 (33)	73 (47)	30 (19)
RA cases Merseyside (239)	28 (12)*	87 (36)**	124 (52)*

*p<0.00001; **p<0.05.
†Social class based on the Office of National Statistics classification of occupations.[7]
‡N = non-manual; M = manual.

Authors' reply

We welcome the letter entitled "Rheumatoid arthritis, poverty, and smoking" in response to our article "Does social disadvantage contribute to the excess mortality in rheumatoid arthritis patients".[1] The importance of smoking as a contributor to the influence of socioeconomic deprivation on mortality is rightly emphasised. However, as Black has pointed out eloquently, smoking alone does not account for the excess mortality seen among lower socioeconomic groups.[2]

We observed a higher mortality rate among patients with rheumatoid arthritis (RA) living in deprived areas relative to those living in affluent areas. Our methodology did not determine the social class of individual patients according to the Office of National Statistics classification of occupations. Nevertheless, whether measured by income, occupation, educational level, social class, or ecological variables such as the Carstairs score, socioeconomic deprivation has been shown to influence health.[3][5] In addition, we observed that there were more patients with RA living in deprived areas relative to the general population in Scotland. Although this may result from a higher prevalence of RA among the lower socioeconomic classes, this conclusion cannot be drawn overtly from our study. Prospective studies of inception cohorts in areas of affluence and deprivation would prove valuable in determining the epidemiology of RA.

In our cohort 40% of the most affluent group (Carstairs 1 and 2), 45% of Carstairs 4, 5, and 6, and 65% of the most deprived group (Carstairs 6 and 7) were current smokers; figures much higher than the 1996 census figures of 12% and 41% for social classes 1 and 4 respectively. This difference may reflect the fact that our patients were recruited a decade earlier (1984–85), but there are also social/cultural differences between Scotland and the United Kingdom as a whole. The prevalence of smoking in Scotland from the Scottish Health Survey 1995[6] was 23% in social classes 1 and 2 and 49% in social classes 4 and 5.

Although differences in mortality rates among patients with RA according to socioeconomic deprivation can be explained, in part, by differences in the prevalence of smoking, the observed influence of deprivation on function in RA is less readily accounted for by smoking.[7] Functional ability is an important outcome measure in RA and is a predictor of mortality in this disease.[8]

The Scottish Health Survey 1995 showed that there were differences according to social class in other important determinants of health, including diet, alcohol consumption, obesity, hypertension, lung function, fibrinogen levels, general health perception, and psychological status. Further research is required to establish the relative importance of these and other factors in determining the influence of socioeconomic deprivation on outcome and mortality in RA and other chronic diseases. The factors which can be modified most effectively to reduce the inequalities in health outcome also require investigation.

If our findings are supported by further studies, socioeconomic status of populations should influence resource allocation. In addition, these important factors should assist rheumatologists when deciding which patients with RA should receive more intensive, multidisciplinary intervention.

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Diagnostic evaluation of classification criteria for RA and reactive arthritis

We read with interest the recent article by Hülsemann and Zeidler,[1] in which the 1987 American College of Rheumatology (ACR) classification criteria for rheumatoid arthritis (RA) were evaluated for their ability to identify patients with a clinical diagnosis of RA among 217 patients referred to an early arthritis clinic. The authors concluded that the 1987 ACR criteria can be used to make a diagnosis of RA in this setting.

In this study, the "gold standard" against which the criteria were tested was an "expert diagnosis" made by one of the authors when the patient was first seen (within one year of symptom onset). However, the main difficulty facing the rheumatologist for patients with early disease is that patients who ultimately develop RA appear clinically similar to those who have self limiting disease or other forms of inflammatory arthritis. It is therefore too early to make an accurate diagnosis at this stage. More importantly, RA is a heterogeneous disease with a prognosis which varies from complete symptom remission to severe disability. Therefore simply categorising patients into those who do and do not have "RA" is not necessarily important when considering which patients require early treatment. Although the authors made a clinical diagnosis without using the classification criteria, it is likely that the diagnoses were informed by their knowledge of the individual components of the criteria. Therefore the high sensitivity (90%) they reported means that most of the patients with a clinical diagnosis of RA will have had seropositive, erosive, polyarticular disease with hand involvement. Whereas we have no problem in recognising these patients as having RA, it represents only one end of the spectrum. The proportion of patients with "undifferentiated

arthritis" in this study is high (54%), though this has been reported in other series.[1][1] It is likely that many of these patients have atypical RA which may still require treatment with disease modifying antirheumatic drugs. Further, in early disease, patients often do not satisfy some of the criteria (nodules, erosions) which are features of established RA. We therefore think it is misleading to imply that patients who do not satisfy the 1987 ACR criteria (a) do not have RA; and (b) do not require early, aggressive treatment.

We recently evaluated the performance of the 1987 ACR criteria in an unselected cohort of 486 patients newly presenting with inflammatory polyarthritis to the Norfolk Arthritis Register.[4] We considered the practical question of whether the criteria could identify which patients would have a poor prognosis after three years as assessed by (a) persistent synovitis; (b) functional disability and (c) radiological erosions. Although we applied the criteria in a number of different ways, we found they had a low ability to discriminate between patients who developed persistent, disabling, and erosive disease and those who did not. For example, applying the criteria in the traditional "list" format, the positive predictive value for erosions was only 45% and the negative predictive value 67%. In practical terms, this means that 33% patients who did *not* satisfy the criteria developed erosions. However, given the fact that the 1987 ACR criteria were developed to distinguish between hospital attenders with established RA and patients with other musculoskeletal conditions, and were never intended to be used as diagnostic criteria, it is not surprising that they do not perform well in this setting.[3]

Finally, we wish to point out that the proportion of patients who satisfy the 1987 ACR criteria is highly dependent on how the criteria are applied. For example, in our study, the proportion of patients who satisfied the criteria at one year of follow up varied from 28% if applied "cross sectionally" (on the day of assessment) to 61% if applied "cumulatively" (each criterion satisfied if "ever" positive). Further difficulties are likely to be encountered using incomplete data ascertained from case note review. It is therefore more appropriate in a group with early synovitis to assess the criteria applied longitudinally at follow up, rather than simply at baseline.[6] In the study by Hülsemann and Zeidler we were given no information about how or when the criteria were applied apart from that they were applied "retrospectively".

We agree with Hülsemann and Zeidler that there is a need to "...differentiate RA as early as possible from the often benign and self-limited forms of undifferentiated arthritis, as there is a need for early treatment of RA". However, we strongly disagree with the use of the 1987 ACR criteria for this purpose. Until we understand more about the pathogenesis of RA, clinicians will have to rely on clinical judgment and the presence of poor prognostic factors to make decisions about whether to treat aggressively patients presenting with early disease.

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MATTERS ARISING

EULAR recommendations for the management of knee osteoarthritis

I welcome the publication of the EULAR recommendations for the management of knee osteoarthritis by the EULAR Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT).[1] However, it is out of date and has been superseded by other more up to date publications such as the recommendations for the medical management of osteoarthritis of the hip and knee by the American College of Rheumatology (ACR) Subcommittee on Osteoarthritis Guidelines[2] and another review of the medical management of osteoarthritis.[3] The newer COX-1 sparing non-steroidal anti-inflammatory drugs, rofecoxib and celecoxib are not discussed in the EULAR recommendations, but they have been included in the other reviews.[2] [3]

My second criticism of the report is that it contains statements such as " SYSADOA... may possess structure modification properties", which is misleading and may lead to widespread use of these drugs in the absence of good evidence.

Although the *Annals of the Rheumatic Diseases* is the official EULAR journal, EULAR reports should be peer reviewed before publication. The ACR recommendations for the management of osteoarthritis of the hip and knee were only accepted in a revised format.[2] Two of the authors of the review in the BMJ[3] are also coauthors of the EULAR report.

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Authors' reply

We thank Dr Jawad for his interest in the EULAR recommendations.[1] The fact that the literature search ended at December 1998, excluding evidence based discussion of COX-2 inhibitors, is clearly highlighted and discussed in the paper. Unfortunately, a finite end point needs to be set on any thorough evidence based review, and even the ACR document was of necessity four months out of date by the time it was published. However, the important strengths of the EULAR document compared with the ACR report are:

1 It used a structured evidence based format rather than just consensus statements.

2 It had input from 23 experts from 12 countries, as opposed to four expert Americans.

3 It undertook additional analyses on published trials to calculate effect sizes and numbers needed to treat where sufficient data were available (that is, it provided new data).

4 The search included surgery and treatments such as topical non-steroidal anti-inflammatory drugs, which are not licensed in the USA and which were excluded from the ACR paper.

5 It provided data on the "epidemiology" and quality of clinical trials in knee osteoarthritis (OA) and highlighted important questions that (still in 2001) need to be addressed by future research.

Unlike the review by Walker-Bone and three other coauthors from Southampton,[3] the EULAR report encompassed both medical and surgical treatments, and carefully examined the literature in response to specific clinically relevant questions rather than in an attempt to produce a pragmatic algorithm. We also calculated effect sizes rather than estimates of efficacy, and did not extrapolate treatment data for the knee (the site to which most data relate) to other joint sites. The difference in outcome from these two contrasting approaches is shown, for example, by the recommendation in the *BMJ* report to consider intra-articular steroid injection only if an effusion is present, whereas the EULAR committee concluded there is no clear evidence to justify a knee effusion as a predictor of response to such treatment.

The qualified statement that "SYSADOAs may possess structure modification properties, but more studies, using standardised methodologies are required" was based on available evidence and made without prejudice. To introduce bias by ignoring published reports on such treatments would indeed be misleading and against all the ethos of evidence based practice.

Although this was a paper that was drafted, edited, and remodified by 21 European experts in OA, the manuscript was peer reviewed by two additional referees after its submission to the *Annals*.

The committee are delighted with the growing interest in the management of OA and that three contrasting reviews were published on the topic in 2000. We, of course, realise the current interest in highly selective COX-2 inhibitors. Importantly, however, the three reports fully concur with respect to the important elements of OA management—the non-pharmacological interventions. They also all agree that paracetamol remains the oral analgesic to try first and, if successful, is the preferred long term oral analgesic.

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Coffee consumption, RF, and the risk of RA

We read with interest the paper entitled "Coffee consumption, rheumatoid factor, and the risk of rheumatoid arthritis",[1] in which an association between coffee consumption and the presence of a positive rheumatoid factor (RF) in healthy subjects was reported. The authors also observed an increased risk of developing seropositive rheumatoid arthritis (RA), particularly in people who were lifelong non-smokers and drinking four cups of coffee or more. Cigarette smoking seemed to be the single most important risk factor for the development of a positive RF in healthy subjects and was strongly associated with coffee consumption. In view of this, however, it seems surprising that there is no additive risk noted for the development of seropositive RA in people who were heavy smokers and also habitual coffee drinkers.

We suggest that the observed risk for developing seropositive RA in people who have never smoked and who consume more than four cups of coffee is possibly confounded by exposure to passive cigarette smoke. For example, coffee in Finland is often consumed in coffee houses, which are prone to be particularly smoky environments, and coffee drinkers are likely to mix with other coffee drinkers, who are statistically more likely to be smokers.

The risk observed for developing seropositive RA in this study is modest for subjects smoking fewer than 14 cigarettes a day (relative risk 1.28, CI 0.63 to 2.57). At face value, it would seem unlikely that passive smoking could play a role in the development of seropositive RA. However, a Chinese study noted that active cigarette smoking was a moderate risk factor for nasopharyngeal cancer (NPC) (OR=1.28, 95%CI 1.02 to 1.61). Despite this, it was observed that women who were lifelong non-smokers had a strong and statistically significant positive association between NPC risk and exposure to substantial passive smoke as a child or as an adult.[2] Interestingly, those with NPC had an increased risk of a family history of NPC compared with controls. Subjects with RA who are lifelong non-smokers would seem to have a greater genetic predisposition to RA, indeed we have found a strikingly high prevalence of a positive family history of RA in a first or second degree relative in these subjects (54%).[3] Possibly, in such genetically "predisposed" subjects passive smoke may induce rheumatoid factor production by a mechanism similar to that in people who are active smokers. The presence of a positive RF in a healthy person is important, as this increases the risk sevenfold for the development of seropositive RA.[4]

A second potential mechanism by which passive smoke inhalation might predispose subjects to RA is by changing the local environment of the nasopharynx. Both the pneumococcus and meningococcus bacteria are carried in the nasopharynx and both invasive pneumococcal and meningococcal disease are seen significantly more often in passive

smokers.[5][6] It is interesting to note that NPC is associated with the Epstein-Barr virus,[7] one of a number of viruses which have been implicated in the pathogenesis of RA.[8] Therefore, passive smoke might potentially predispose subjects to RA as a result of changes within the nasopharynx resulting in antigenic stimulus by a virus that triggers RA.

We agree with the authors that new information is urgently needed about any factor associated with the risk of RA. In view of emerging data highlighting smoking as an important environmental risk factor for the development of seropositive RA,[3][9] and also this study by Heliovaara *et al*, we propose that passive smoking should be considered as a potential candidate factor for the development of RA.

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Authors' reply

It is not easy to measure passive smoking. Nevertheless, determinations of serum cotinine and thiocyanate recommended for the detection of indirect exposure to tobacco smoke have been shown to indicate exposures of recent occurrence.[1][2]

To test the assumption put forward by Drs Hutchinson and Moots we studied coffee consumption for its associations with serum cotinine and thiocyanate concentrations in a sample of men who had participated in the Mini-Finland Health Survey and served as an age matched control group in a nested case-control study of lung cancer.[3] A modification of the Nicotine Metabolite RIA kit method (Diagnostic Products Corporation, Los Angeles, USA) was used to determine serum cotinine concentrations.[4] Serum thiocyanate was determined by the spectrophotometric ferric nitrate method.[5] Of the total of 158 men in the sample, 39 reported current smoking, and 10 others had serum cotinine ≥200 mg/l or thiocyanate ≥20 nmol/l, suggesting direct exposure to tobacco. Exclusion of these men left 109 non-smokers for the final analyses.

No significant association was seen between the number of daily cups of coffee and serum cotinine concentration (age adjusted $r=0.08$, $p=0.38$). Contrary to the assumption of Drs Hutchinson and Moots, there was a negative correlation between serum thiocyanate level and coffee consumption (age adjusted $r=-0.21$, $p=0.03$). The findings are in agreement with our impression that in Finland coffee is not consumed to any greater extent in notably smoky environments. Rather, coffee consumption is confined to breakfast at home and coffee breaks both during working hours and leisure time.

On the basis of these preliminary results it seems unlikely that passive smoking would have correlated strongly enough with coffee consumption to explain the close associations between coffee consumption and the occurrence of rheumatoid factor (RF) and the risk of rheumatoid arthritis (RA) in our study. Nevertheless, our results are far from being conclusive. Whatever the links between coffee consumption, RF, and RA, we agree with Drs Hutchinson and Moots that passive smoking should be considered a potential risk factor for RA.

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LETTERS TO THE EDITOR

Polyarteritis nodosa complicated by thrombotic thrombocytopenic purpura

A 56 year old woman was diagnosed with polyarteritis nodosa (PAN) in June 1998

based on the presence of fibrinoid necrosis and infiltration of polymorphonuclear cells into medium and small sized arteries on a skin biopsy specimen. She presented with erythema on her arms and legs, with fever and body weight loss. Tender masses were palpable on her right abdomen. Small erythematous lesions and livedo reticularis were seen on the arms and legs.

Laboratory investigation on admission disclosed anaemia (haemoglobin 73 g/l) and leucocytosis (22.5×10^9/l) consisting mainly of neutrophils (85%). Creatinine clearance was 39 ml/min. Serological examination showed raised levels of C reactive protein (88.6 mg/l). Serological tests for syphilis, hepatitis B virus antigen, and antibody for hepatitis C virus were negative. A high titre of myeloperoxidase antineutrophil cytoplasmic antibody (MPO-ANCA) (201 EU) was detected in her sera. An abdominal computed tomography scan showed bilateral perirenal haemorrhages.

The patient was treated with 1000 mg of methylprednisolone for three successive days, followed by 500 mg cyclophosphamide intravenously. Plasma exchange was performed, also. Within three days, treatment had reduced the body temperature to normal and lowered the C reactive protein concentration (<10 mg/l).

The patient rapidly developed thrombocytopenia on the fifth day after admission to hospital, and the lowest platelet count was 15.0×10^9/l on the eighth day after admission. She became unconscious, and showed features of haemolytic anaemia and renal failure. A provisional diagnosis of disseminated intravascular coagulation was made because of thrombocytopenia, a low concentration of fibrinogen (1.6 g/l), and a high fibrinogen and fibrin degradation product level (22.6 µg/ml). We treated her with nafamostat mesilate (200 mg/day) and infusion of fresh-frozen plasma. This treatment produced a marked increase in fibrinogen concentration but failed to improve the platelet count. Further laboratory tests showed fragmented red blood cells in peripheral blood. A diagnosis of thrombotic thrombocytopenic purpura (TTP) was established based on these findings. The patient was treated with plasma exchange with 2700 ml fresh-frozen plasma.

Within one week of treatment the platelet count returned to normal and consciousness level improved dramatically. However, renal failure was irreversible and she continued to undergo dialysis. Unfortunately, the patient died from haemorrhage from a duodenal ulcer. Necropsy findings included small and medium sized polyarteritis in the kidney, uterus, pulmonary hilum, hepatic hilum,
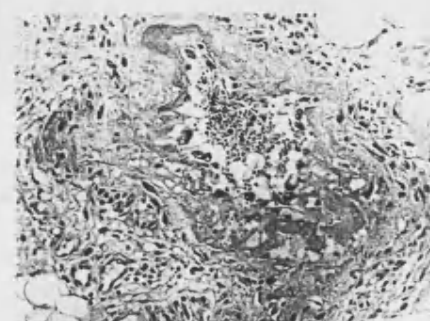

Figure 1　Necropsy finding of ileum end. Note fibrinoid necrosis of medium sized artery. Haematoxylin and eosin stain. Magnification ×400.

Serum complexes of immunoglobulin A-α1 proteinase inhibitor in rheumatoid arthritis: Association with current cigarette smoking and disease activity

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Abstract

Objective

To investigate whether high levels of serum immunoglobulin A-α1 proteinase inhibitor (IgA-α1PI) complexes are primarily associated with cigarette smoking or the rheumatoid arthritis (RA) disease process itself.

Methods

A case-control study consisting of 231 RA cases and 83 healthy hospital workers. A smoking history was taken for the study groups. The serum IgA-α1PI complex levels (arbitrary units, au) were determined using a sandwich ELISA. Erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF) measurements were recorded in each of the RA cases. The serum complex levels were compared between RA cases and controls matched for smoking history and between smokers and non-smokers in the RA cases and controls.

Results

Mean serum IgA-α1PI complex levels were significantly higher in RA current smokers than in non-smoking RA patients (17.4 v 11.9 a.u., p = 0.0001). Similarly, mean serum complex levels were significantly higher in control current smokers than control non-smokers (18.8 v 11.5 a.u., p = 0.003). Seropositive RA cases had significantly higher complex levels than seronegative cases. Patients with erosive disease had higher levels than non-erosive patients, although significance was lost after correction for current smoking and RF positivity. There was an association between ESR and serum IgA-α1PI complex levels which was independent of current smoking. Overall, there was no significant difference in complex levels between RA cases and controls after correction for current smoking.

Conclusion

Raised serum IgA-α1PI complex levels are associated with current smoking in both RA and healthy controls. ESR levels in RA patients are also associated with serum complex levels independently of current smoking. Our data suggest that high IgA-α1PI complex levels can be generated either as a result of current smoking, or by an active disease process in RA patients.

Key words

IgA-α1PI, rheumatoid arthritis, smoking, rheumatoid factor.

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Introduction

Rheumatoid arthritis (RA) is a heterogeneous disease, with a disease spectrum ranging from a persistent inflammatory, but non-erosive joint disease to a rapidly progressive erosive joint disease (1). Rheumatoid erosions are associated with increased proteinase activity within the joint (2). In particular, the matrix metalloproteinases (MMPs), a class of zinc-dependent proteinases responsible for the metabolism of extracellular matrix proteins (3) are thought to play an important part in joint destruction (4). Their activity is precisely controlled by tissue inhibitors of MMPs (TIMPs) (5). However the inhibitory activity of TIMPs may be destroyed by other proteinases within the joint; e.g. TIMP 1, an important inhibitor of the specific MMPs that cause rheumatoid joint damage (6), is destroyed by neutrophil elastase (NE) (7), a serine proteinase, which can also degrade articular cartilage (8). NE also activates latent stromelysin-1 (9), an important MMP involved in joint destruction (10).

Neutrophil elastase is inhibited principally by the major serum protein α1-proteinase inhibitor (α1PI) (11), a potentially important inhibitor of rheumatoid joint damage.

The reactive centre of α1PI is crucial to its ability to inhibit NE (12). This reactive site contains a methionine group that is rapidly oxidised and therefore inactivated by potent oxidants such as hypochlorous acid (reviewed in 13). Hypochlorous acid can be produced by a neutrophil enzyme, myeloperoxidase (MPO), in combination with hydrogen peroxide (H_2O_2) via the MPO - H_2O_2 - chloride system (14). We have demonstrated that oxidation of α1PI by this system promotes binding to immunoglobulin A and the formation of IgA-α1PI complexes (15). We have observed that seropositive, erosive and nodular RA cases have significantly higher serum IgA-α1PI complex levels than both seronegative RA cases and healthy controls (16). We have also found that elastase inhibitory activity of α1PI is considerably reduced in IgA-α1PI

complexes isolated from RA serum (15).

Recently it has been suggested that cigarette smoking is an important trigger factor for the development of erosions in RA (17-20). The way in which smoking influences RA severity is unclear at present, although it may have direct effects on the disease process by inducing and/or increasing production of rheumatoid factor (RF), or by producing alterations in the immune system (21-26). Cigarette smoke is abundant in free radicals and can inactivate α1PI (reviewed in 13). Accordingly we hypothesise that cigarette smoking is associated with the high serum levels of IgA-α1PI complex observed in RA, and suggest that individuals with high complex levels may be more susceptible to erosive joint disease. To test this hypothesis we have compared serum levels of IgA-α1PI complexes in smoking and non-smoking RA cases and examined the relationship with erosive disease. We have also examined levels of these complexes in smoking and non-smoking healthy controls to determine whether complex formation is primarily associated with cigarette smoking or the RA disease process itself.

Methods

Study design

This study was a case-control study. Patients and controls gave informed consent and participated in the study. The study was approved by the ethics committees in each participating centre.

Patient and control population

Adult outpatients with RA (age range 19-88) all fulfilling the ARA criteria for RA (27) and with a clinical diagnosis of RA attending two hospitals in Meseyside, St. Helens Hospital and University Hospital Aintree were recruited for the study. The 231 RA patients were selected to represent a cross-section of rheumatoid disease to test our hypothesis and therefore approximately equal numbers of smokers, ex-smokers and life long non-smokers

Table I. Demographic and clinical characteristics of RA patients (n = 231) and controls (n = 83).

	RA			Controls		
		n	%		n	%
Median age, yrs (range)	61.0	(19 -88)		44.0 (19-75)		
Median age of onset, yrs (range)	49.0	(18 - 82)				
Disease duration, yrs (range)	12.0	(1 - 52)				
Median ESR, mm/hr (range)	31	(5-126)				
Female		173	75		60	72
Current smoker		75	33		45	54
Ex-smoker		72	31		9	11
Never smoked		84	36		29	35
Rheumatoid factor positive		169	73			
Erosive disease		151	65			

Table II. Influence of smoking on IgA-α1PI levels (mean ± SD) in RA patients and healthy controls

		Never	Smoking status Ever	Past	Current
RA		n = 84	n = 147	n = 72	n = 75
		11.9 (16.1)	15.1 (16.2)*	12.8 (13.2)	17.4 (18.4) †
Controls		n = 29	n = 54	n = 9	n = 45
		11.5 (5.9)	17.6 (10.3)§	11.8 (2.9)	18.8 (10.9)#

Multiple regression analyses with complex level as the dependent variable. The p values represents the significance of each smoking variable compared with never smoking.
* p = 0.01 (corrected for sex and disease duration).
† p = 0.0001 (corrected for sex and disease duration)
§ p = 0.002 (corrected for sex).
# p = 0.003 (corrected for sex).

were recruited. The patients' age and age of disease onset were recorded. A case note examination of the RA cases was undertaken to determine both rheumatoid factor status and for radiological evidence of erosive joint disease. Seropositive patients were defined as seropositive if previously the rheumatoid latex test had been positive at a titer ≥ 1:40. RA patients with active infection or chronic infective processes such as bronchiectasis were excluded from the study. The demographic and clinical characteristics of the RA patients and controls are shown in Table I. The 83 control cases were recruited from healthy hospital workers.

A comprehensive past and current smoking history was taken from the time of inclusion in the study. Cigarette consumption was quantified in pack years (PY). One PY is equivalent to 20 cigarettes smoked per day for one year. A current smoker was taken to be a smoker of at least 1 cigarette per day

for 1 year. An ex-smoker was defined as an individual who had stopped smoking for at least 3 months.

IgA-α1PI complex levels and laboratory assessments
A single 10 ml venous sample was taken from each case. The blood was centrifuged for five minutes and the plasma stored at -70°C. The IgA-α1PI complex levels (arbitrary units, au) were determined using a sandwich ELISA. The methodology has been described previously (16). A reference plasma sample from an individual with high levels of IgA-α1PI complex was used to obtain a standard curve from which arbitrary units (a.u.) were obtained. The erythrocyte sedimentation rate (ESR) was measured in the RA cases only.

Statistical analysis
The relationships between IgA-α1PI complex levels and smoking status,

erosive RA and RA disease activity were examined by multiple regression analyses with correction for the independent variables, age, gender, and disease duration where appropriate. In cases where variables were not normally distributed they were transformed to normality by using the log values. Multivariate logistic regression analysis was used to determine the variables most closely associated with erosive RA. All analyses were carried out using the Number Cruncher Statistical Package for Windows (NCSS v.6.0.4).

Results
Influence of smoking on IgA-α1PI complex levels in RA patients and healthy controls
RA patients who had ever smoked had significantly higher IgA-α1PI complex levels than those who had never smoked (p = 0.01) (Table II). Division of ever smokers into past and current smokers revealed that the increased complex levels were associated only with the current smokers (p = 0.0001). The RA past smokers had similar complex levels to the RA group that had never smoked. Similar results were found in the healthy control population, the highest complex levels again being found in current smokers (p = 0.003). No significant differences in complex levels were found between non-smoking RA patients and non- smoking controls, or between RA patients and controls who smoked. Correction for age made no difference to these analyses.

We found no relationship between the number of cigarettes smoked per day and complex levels in the RA current smokers. However, in all patients who had ever smoked a weak association was found between pack years smoked and complex levels (p = 0.05, after correction for sex and disease duration). No association was found in the healthy control group.

Relationship between IgA-α1PI complex levels and seropositivity in RA patients
Complex levels in RF+ RA patients (n = 169) were significantly higher than in

Table III. Multiple regression analysis to investigate the association of ESR, seropositivity and current smoking with IgA-α 1PI levels in RA patients.

Independent variable	Regression coefficient	Standard error	p value
RF (+/-)	0.424	0.134	0.002
Current smoking	0.366	0.125	0.003
Log ESR	0.182	0.072	0.011

Multiple regression model with log IgA-α1PI complex level as the dependent variable. The model was corrected for sex and disease duration by including these covariates with the independent variables. F-ratio 7.90, $p < 0.0001$.

RF- patients (n = 62) (16.1 v 8.8 a.u., $p < 0.0001$, after correction for sex and disease duration). Multiple regression analysis demonstrated that seropositivity was associated with higher complex levels independently of current smoking after correction for sex and disease duration. In this model the association of complex levels with RF positivity was stronger ($p = 0.0001$) than that with current smoking ($p = 0.013$).

Relationship between IgA-α1PI complex levels and ESR levels

We found a significant association between IgA-α1PI complex and ESR levels in the total RA population ($p = 0.004$, after correction for sex and disease duration by multiple regression analysis). This association remained significant after further correction for current smoking and seropositivity (Table III).

Table IV. Multivariable determinants of erosive disease in RA.

Variable	Regression coefficient	Standard error	p value
Current smoker	0.836	0.422	0.043
RF (+/-)	2.237	0.423	< 0.0001
log IgA-α 1PI	0.336	0.227	0.14
Duration	0.141	0.029	< 0.0001
Female Sex	0.852	0.422	0.044

Logistic regression analysis with the presence or absence of erosions as the dependent variable. Chi-square for the model with 5 degrees of freedom = 103.41 ($p < 0.0001$).

Relationship between IgA-α1PI complex levels and erosive disease

Complex levels in patients with erosive disease (n = 151) were significantly higher than in patients with non-erosive disease (n = 80) (15.9 v 10.5 a..u., $p = 0.001$ after correction for sex and disease duration). However, significance was lost in a multivariate logistic regression analysis which also included current smoking and RF positivity as independent variables (Table IV).

Discussion

This study has shown that RA patients who are current smokers have significantly higher IgA-α1PI complex levels than non-smoking RA patients. However, we have also found that healthy individuals who smoke have elevated complex levels which are comparable with those found in RA patients who smoke. This would suggest that high complex levels in RA smokers do not occur as a result of RA per se, but are produced principally as a result of cigarette smoking. Nevertheless, we also found that a marker of RA disease activity (ESR) was associated with serum complex levels independently of current smoking. These data suggest that high serum complex levels can be generated either as a result of current smoking, or by an active disease process in RA patients. We hypothesise that this complex formation is induced through oxidative stress that is generated either by smoking, or the rheumatoid disease process itself.

We confirmed previous findings of increased IgA-α1PI complex levels in RF+ patients (16). In addition we found that the association of complex levels with seropositivity was independent of current smoking. This does not appear to be due to any effect of RF on the IgA-α1PI assay since we have shown previously that RF does not interfere with this assay (16). Although both RF and IgA-α1PI are associated with current smoking they appear to be produced independently, and they demonstrate differences in their dependence on the overall smoking history. For example, there was only a very weak as-

sociation between pack years smoked and complex levels in the RA group. This is in contrast to studies that have observed a strong association between pack years smoked, increased RF concentration and joint damage (18,19). Furthermore, RF production, unlike high serum levels of IgA-α1PI complex, may persist in heavy ex-smokers. Wolfe observed that former heavy smoking RA patients had similar RF levels to current heavy smokers (18) and a recent study observed that RF persisted despite the cessation of smoking in healthy individuals (28). Our data suggest that the continual generation of IgA-α1PI complexes may depend on continuing oxidative stress and that high serum levels of these complexes return to normal once the source of the oxidative stress (i.e. smoking) has been stopped.

The mechanisms by which current smokers with RA are more likely to develop erosive disease than non-smokers with RA are not known. Wolfe (18) has recently demonstrated that smoking can induce both erosive and nodular rheumatoid disease independently of rheumatoid factor. Our data confirm an independent association of RF and current smoking with erosive disease, although RF has by far the strongest association. We also found that IgA-α1PI complex levels were higher in patients with erosive disease, although significance was lost after correction for RF and current smoking. Nonetheless, it is possible that the effects of smoking may be mediated in part through increased IgA-α1 PI complex formation. Cigarette smoke contains an abundance of free radicals and has been observed to oxidise and inhibit the function of α1PI (13). The high complex levels observed in some RA patients who currently smoke may partly explain the association of current smoking with the development of erosive RA. In these patients significant joint destruction could possibly occur through a process involving increased neutrophil elastase activity. Neutrophil elastase may directly degrade cartilage or indirectly upregulate MMP activity by inhibition

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of a tissue inhibitor of MMPs (TIMP 1).

Our study did not directly address whether a reduced capacity to inhibit NE predisposes to rheumatoid joint damage. Some erosive RA patients who were currently smoking did not have raised complex levels. Thus, in these patients joint damage does not appear to be associated with oxidation of α1-PI, although oxidative stress may still play a role (29). Free radicals may activate MMPs, cysteine and other serine proteinases and cause direct damage to cartilage (30). Equally, free radical mediated alteration of IgG may stimulate the formation of immune complexes with RF antibody, thereby promoting tissue damage during rheumatoid inflammation (31, 32).

In conclusion, levels of serum IgA-α1PI complex are significantly raised in both healthy smokers and RA smokers. Serum IgA-α1PI complex levels correlate with ESR levels independently of current smoking or RF positivity in RA. These data suggest that high IgA-α1PI complex levels can be generated either as a result of current smoking, or by an active disease process in RA patients. Further studies are needed to determine the prognosis of RA cases with raised levels of serum IgA-α1PI complexes and the possible genetic factors predisposing these individuals to increased production of IgA-α1PI complexes.

Acknowledgements

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high-titre IgM RF (odds ratio 2.78) than individuals who had never smoked and that individuals smoking fewer than 15 cigarettes per day had a very modestly increased risk of developing seropositive RA (relative risk 1.28) compared with the much higher risk of smoking 25 or more cigarettes per day (relative risk 3.43). They also observed that former smokers were more likely to develop seropositive RA than lifelong non-smokers (relative risk 1.76). These data suggest that RF production is associated with high-intensity cigarette smoking and that, once smoking has induced RF production, this process continues despite the cessation of cigarette smoking. In their study, Masdottir et al. observed an association between pack-yr smoked and IgM and IgA RF in female patients, and likewise Wolfe [5] observed a significant association between an increased concentration of IgM RF and pack-yr smoked in RA, independent of the smoking status of the patient.

We propose that, in healthy individuals, as in RA patients, heavy prolonged smoking (but not smoking per se) is associated with RF production and the subsequent development of seropositive RA. Indeed, we have reported a striking association between heavy smoking (40–50 pack-yr) and RA, but only a modest association for ever having smoked [6]. Although cigarette smoking has been consistently associated with RA, a study of pre-menopausal RA patients observed an apparent protective effect for current smoking [7]. However, this might be explained by their young age (20–50 yr); the majority of women studied would not have accumulated the sufficiently high exposure required to provide the susceptibility factor. In keeping with this, we have observed that lifelong non-smoking RA cases develop RA at a significantly younger age than RA cases smoking at disease onset [6], and we note that a similar trend is observed by Masdottir et al. [1].

Regarding heavy smoking and increased RA disease severity, we suggest that the association observed by Masdottir et al. [1] is principally the result of comparing seropositive RA with seronegative RA. We note that in their study group half the women were seronegative; the majority of this group are likely to have never smoked [8]. To investigate the role of heavy smoking in RA, they compared those who had smoked less than 20 pack-yr with those who had smoked more than 20 pack-yr. However, as they included women who had never smoked in the group who had smoked less than 20 pack-yr, this group was biased because as many as 51% had actually never smoked. Therefore, heavy smokers were effectively compared with lifelong non-smokers rather than modest smokers. As suggested above, heavy smoking is associated with the development of RF, predisposing individuals to seropositive rather than seronegative RA. The differences in severity observed between the two groups studied may simply reflect the clinical differences that exist between seropositive and seronegative RA. Saag et al. [8] compared lifelong non-smokers with heavy smokers and observed an increased risk of seropositivity, erosions and nodules. Wolfe [5] did observe

Cigarette smoking and severity of rheumatoid arthritis

SIR, We read with great interest the paper by Masdottir et al. [1] reporting that women with rheumatoid arthritis (RA) and considerable exposure to cigarette smoke were more likely to have severe disease and nodules than women who smoked modestly. However, we propose that heavy prolonged smoking is also associated with the development of seropositive RA rather than simply disease severity (in terms of joint damage) and that the development of rheumatoid factor (RF) in heavy smokers partly underlies this important association.

The presence of RF in healthy individuals is perhaps more significant than generally understood, as persistence of both IgM and IgA RF is associated with a seven-fold risk of developing seropositive RA [2]. Of the known environmental risk factors associated with the development of RF in healthy individuals, cigarette smoking is by far the most important [3]. Whilst the association between pack-yr smoked and the presence of RF has not been studied in healthy individuals, healthy current smokers are significantly more likely to be positive for both IgM and IgA RF than non-smokers [4]. Furthermore, Heliovaara et al. [3] demonstrated that healthy individuals who had previously smoked had an increased risk of developing

an association between increasing pack-yr smoked and increasing joint damage, but this was only apparent at a smoking intensity of above 40 pack-yr.

If there is a true dose response with pack-yr smoked and RA joint disease, a comparison between those smoking between 1 and 20 pack-yr and those who have smoked more than this should reveal a difference in severity. We would be interested to know if this is the case.

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RA in the population studied overall. However, following stratification for gender, males with sporadic RA were older at disease onset than male probands with familial RA [median (range) 57 (24–78) vs 50 (24–61) yr, $P = 0.03$]. This was not the case regarding female RA cases.

We would suggest that a history of current cigarette smoking could account for the difference in age of onset observed between males with familial and sporadic RA. It is well established that cigarette smoking is a particularly important risk factor for the development of RA, particularly in men [2], and that heavy cigarette smoking, but not smoking per se, is also associated with RA in women [3].

To determine if cigarette smoking at the time of onset of RA influences the age of onset of familial and sporadic RA, we considered RA patients smoking at the time of disease onset and those who had never smoked. Three hundred and sixty unrelated RA patients (age range 28–87 yr) satisfying the above criteria attending rheumatology clinics in two Merseyside hospitals and fulfilling the 1987 American Rheumatism Association (ARA) criteria for RA were studied further [4].

The patients' age, age of disease onset, family history of disease (first- or second-degree relatives with a history of RA) and smoking history were recorded. Considering those patients with familial ($n = 90$) and sporadic RA ($n = 134$) who were smoking at the time of disease onset, there was a striking significant difference in the age at onset when comparing these two groups. The familial RA patients' median age of onset was earlier than in the sporadic RA patients [40 (18–72) vs 50 (20–75) yr, $P < 0.00001$]. These differences were observed in both male and female cases (data not shown). On the other hand, comparing patients who had never smoked, the median age of onset of disease was similar in both groups [familial RA ($n = 70$) 44 (18–75) yr and sporadic RA ($n = 66$) 46 (19–74) yr, $P = 0.6$]. These findings are summarized in Table 1.

Considering the smoking sporadic RA cases, there was a trend ($P = 0.06$) for the age of onset to be later than the never smoked sporadic RA cases [50 (20–75) vs 46 (19–74) yr]. It is possible that for the smoking sporadic RA cases, cigarette smoking is their principal risk factor for the development of their disease and that this risk is only evident following prolonged exposure to cigarette smoke, as is the case with other smoking-related diseases such as lung cancer [5] and pulmonary

The influence of current cigarette smoking on the age of onset of rheumatoid arthritis (RA) in individuals with sporadic and familial RA

Sir, We read with interest the paper by Radstake et al. [1] entitled 'Familial vs sporadic rheumatoid arthritis (RA). A prospective study in an early RA inception cohort'. The main finding of this study was that sibship size is the only relevant risk factor associated with familial RA. No differences in genotypic and phenotypic characteristics, disease severity or radiological damage were observed when comparing familial and sporadic

Table 1. The influence of current cigarette smoking on the age of onset of RA familial and sporadic disease

RA patient type and smoking history at RA onset	Number	Median age at onset (range)	P value
Sporadic smoker	134	50 (20–75)	<0.000001
Familial smoker	90	40 (18–72)	
Sporadic never smoked	66	46 (19–74)	0.6
Familial never smoked	70	44 (18–75)	

emphysema [6]. This process as a risk factor for RA may only become evident after at least 30 yr of smoking and therefore this particular group present later in life.

The opposite trend, however, was observed in familial RA cases. The smoking RA cases' median age of onset was earlier than in the never smoked cases [40 (18–72) vs 44 (18–75) yr, $P = 0.16$]. It is possible, therefore, that smoking and a particular gene or genes interact to increase the risk of developing RA at an earlier age. In their study, Radstake et al. [1] observed that HLA-DR alleles coding for the shared epitope were similar and not significantly different between patients with familial RA and sporadic RA. However, other possible RA susceptibility genes, such as the alpha 1 proteinase inhibitor Z allele [7], are observed in familial RA [8] as opposed to randomly selected RA [9]. The alpha 1 proteinase inhibitor Z allele interacts significantly with cigarette smoke in diseases such as pulmonary emphysema [10] and this may also be the case in RA. Finally, in view of our findings, we would suggest that the observation of Radstake et al. [1] that disease onset in familial RA cases is earlier than sporadic RA in males, but not females, could be explained by a difference in the prevalence of cigarette smoking between their male and female cases at the time of disease onset.

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Reply

We have the following comments concerning the previous letter [1]. Our study was performed in a well-documented cohort using prospective assessments of disease severity and outcome. We demonstrated that only the sibship size is associated with the risk of familial rheumatoid arthritis (RA) and found that affected males with familial disease were younger at disease onset compared with familial RA females and males with sporadic RA.

In contrast, the above authors [1] performed a cross-sectional study and found that smoking patients with familial RA had an earlier disease onset than those with sporadic RA independent of gender. However, this difference was not present in the non-smoking patients. These authors therefore suggest that a history of smoking could play a role in our population and explain the younger age at disease onset in males with familial RA. Because smoking history was not assessed in our study this cannot be determined.

Nevertheless, it is clear that several environmental and genetic factors, which are still unknown, could play a role in the susceptibility to and/or severity of RA. These potential factors include smoking, pregnancy and hormonal substitution therapy and other socio-economic factors. We do believe that the only way to clarify this question is to perform regression studies and discriminate analysis in well-documented populations considering potential confounders.

The present literature regarding smoking and α-1-antitrypsin deficiency and their influence on RA are subject to possible bias and confounding. Most [2, 3], but not all [4] studies on the influence of smoking show only a marginal increased risk for RA in smokers and are cross-sectional and retrospective in design. Furthermore, most studies on α-1-antitrypsin deficiency are biased by the selection of patients with severe arthritis. Moreover, recent genome-wide linkage studies do not point towards potential susceptibility loci on chromosome 14 in the vicinity of the α-1-antitrypsin gene [5, 6].

Our major criticism of the above-mentioned study is that it is cross-sectional and does not show any inclusion criteria. Another important point is that the definition of familial RA in this study differs substantially from that in ours. The inclusion of both first- and second-degree relatives in the former makes a reliable ascertainment of familial RA of paramount importance. We know from our experience that it is extremely difficult to have a secure ascertainment in this circumstance [6]. The way in which this is acquired in the present study is not clearly addressed.

We do not see a good reason why smoking would influence the age at onset of RA only in familial cases. The authors suggest a role of α-1-antitrypsin deficiency, which seems a very hypothetical statement. We do believe, however, that the proposed relationship

between smoking and age at onset needs further confirmation in larger cohorts.

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